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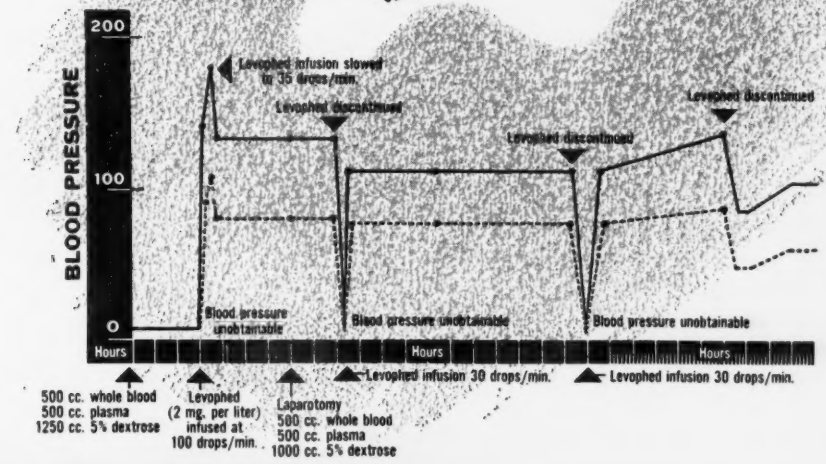

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THE DIGITAL EXPLORATION OF THE VENTRICULAR CHAMBERS OF THE HEART THROUGH A RUBBER DIVERTICULUM*

WILLIAM W. L. GLENN

If access to the ventricular chambers of the heart could be obtained without loss of blood or interruption of the normal functions of the heart, certain congenital and acquired lesions of the interior of the heart might be partially or completely corrected with minimal risk. The present investigation was stimulated by the needs of an infant in severe cardiac failure due to the presence of a ventricular septal defect. The correction of defects of the atrial septum with the use of an open rubber extension of the auricular appendage has recently been reported by Gross and his co-workers.[1] The widespread acceptance by the surgeon of the operation for mitral valvulotomy has been largely due to the fact that there is loss of only a small amount of blood when the mitral valve is approached through the auricular appendage. It occurred to us that perhaps a closed rubber appendage, or what we would prefer to call a diverticulum, could be temporarily attached to the ventricles to permit a similar bloodless approach to the structures within these chambers.

We do not have a definite answer to all the problems which were encountered in the development of this technique; but certain deductions can, however, be made from an experience with 26 dogs, when a diverticulum was sewed onto either the right or left ventricle and where an incision was made through the ventricular wall into the chamber. In 13 of these animals a ventricular septal defect had been made and the diverticulum was used as a method of approach to the ventricular chambers for repair of the defect. The results of these experiments will be reported at a later date.[2]

METHOD

In our earliest experiments an ordinary condom (Trojan) was used as a diverticulum for the right ventricle and a finger cot for the left ventricle. Because of the thinness of the rubber, blood sometimes leaked through the needle holes in the rubber or at the junction of the heart and the diverticulum. We therefore designed a rubber diverticulum (Fig. 1A) which we made from liquid latex (kindly furnished by the Seamless Rubber Company of New Haven) on a simple glass mold. The wall thickness of the diverticulum that we have found to be most useful measures approximately 0.015 inches. The diverticulum is oval in shape, 1.25 inches internal diameter, and 2.5 inches maximal length. A bead about 0.09 inches thick is made at the open end which is rolled over once

* From the Department of Surgery, Yale University School of Medicine. Aided in part by grants from the United States Public Health Service, National Institutes of Health, and the Connecticut Heart Association.

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before the suturing is begun. At the closed end of the diverticulum there are two extensions: a larger one which, when opened, will admit the distal end of the index finger, and a smaller one, which, when opened, will admit a small catheter to deliver a slow heparin drip (10 mgm. per 100 cc. normal saline) or an instrument (Fig. 1B). An area on the ventricle free of major coronary arteries is selected for the point of

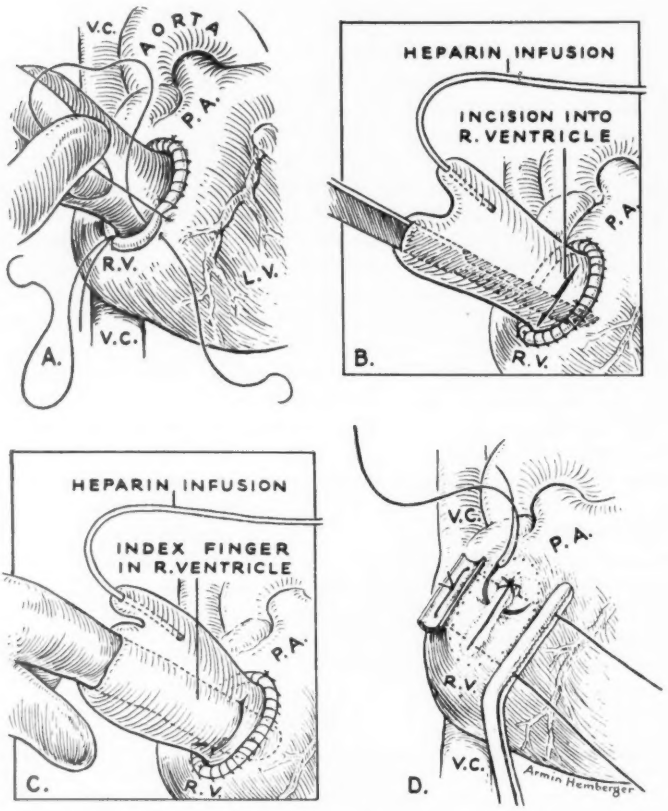

FIG. 1. Method for the digital exploration of the right ventricle through a rubber diverticulum.

suture of the diverticulum. Needle puncture of obvious branches of the coronary arteries is avoided. The diverticulum is fixed to the myocardium by four interrupted 5-0 Deknatel sutures tied firmly with a surgeon's knot and which are then continued around the base of the diverticulum, each suture passing under the rolled-up bead through the rubber and through a moderately small piece of myocardium. It is useless to take broad bites of myocardium with the needle as the thread will cut through excessive muscle as tension is made on the suture line. Between sutures moderate but

firm tension must be kept on the suture material. Occasionally one or two additional interrupted sutures are necessary to obtain hemostasis after the incision in the heart has been made. We have often placed two or three cubic centimeters of fresh blood into the diverticulum and allowed it to clot around the suture line, with the thought that it might prevent leakage of blood, after the heart is opened, through any small puncture hole made by the needle.

A two-centimeter incision in the ventricle is made with the knife in the diverticulum after it is filled with saline solution. The heparin-saline drip is begun at this time. In making the incision, a No. 15 Bard-Parker blade or a No. 34 Beaver blade has been used. The incision is made slowly, the operator watching for a gush of blood into the diverticulum. It is continued slowly after this, care being taken not to cut too deeply and to avoid injuring structures within the heart (Fig. 1B). The knife is then removed as a rubber-shod clamp is placed across the diverticulum about two inches from the heart. By enlarging the hole in the larger extension on the diverticulum, the end of the index finger can be placed inside (Fig. 1C), the rubber-shod clamp removed, and manipulation within the heart carried out. Frequently, the incision has been too small to admit the finger but it can be easily stretched by the finger to the proper size.

To close the incision in the heart a suture of strong silk on a large needle is placed through the myocardium beneath the diverticulum at one end of the incision. It is then passed through a small piece of polyethylene tubing split in half. A similar suture is placed at the other end of the incision and the two sutures are tied over the polyethylene. The two sutures are then drawn tight on the opposite side of the diverticulum and are held with a Kelly clamp or other suitable clamp. This manoeuvre draws the cut edges of the incision in the ventricle together. The diverticulum is then cut off and the incision is closed with interrupted silk sutures (Fig. 1D). After closure of the incision the larger fixation sutures are cut close to the myocardium after removal of the clamp, and the sutures and polyethylene are removed. This method of closure of the incision is similar in principle to those suggested to us by Lindskog and by Harken.

Results

A rubber diverticulum was sewed onto the ventricle in 26 dogs; in 20 on the right side and in 6 on the left. Butyn sulfate, 2 per cent,[3] was instilled into the pericardial sac in many of the animals prior to beginning the suture of the diverticulum to the ventricle. Butyn invariably caused a slowing of the heart and decreased the irritability of the heart to direct stimulation, a feature which is undesirable in the event of cardiac arrest or ventricular fibrillation. In some animals no local anesthetic agent was applied to the myocardium. No animal developed cardiac arrest or ventricular fibrillation as a result of the suturing of the rubber diverticulum to the ventricle. Incision of the ventricle wall invariably caused transient ectopic ventricular beats, but only one animal died immediately following an incision into the cavity of the right ventricle. Just prior to making the incision, this animal—an old dog—was given a supplementary dose of nembutal intravenously. A moderate-to-marked fall in systemic blood pressure occurred in all animals immediately following an incision into the left ventricle. Recovery of the blood pressure could be hastened by the slow intravenous administration of a dilute solution of nor-epinephrine. When the condom was used as the diverticulum on the right side, a marked fall in blood pressure sometimes

occurred, probably due in part to the fairly large volume (40-50 cc.) of blood which filled the diverticulum, and in part due to the distensible thin rubber which absorbed some of the effective pressure created by the contraction of the ventricle. There was usually a moderate fall in blood pressure following incision into the right ventricle when the smaller and thicker-walled diverticulum was used (Fig. 2). Bleeding around the junction of the diverticulum and the ventricle was frequent when the thin-walled diverticulum was used. Interrupted sutures were placed to control this bleeding. Using the thicker rubber diverticulum, bleeding has been rare and only occasionally has a single additional suture been required following incision into the right ventricle. As one might expect from the higher pressures developed, perfect hemostasis at the suture line was somewhat more

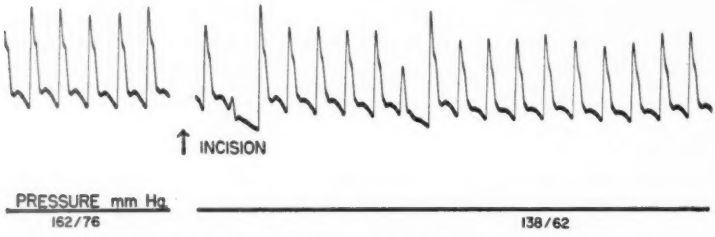

FIG. 2. Opening of right ventricle. Femoral artery pressures.

difficult to attain with the left ventricle opened. With the aid of an occasional extra suture and clotting of blood at the junction of the rubber and the myocardium, perfect hemostasis could be attained at the suture line with an intraventricular systolic pressure in excess of 150 millimeters of mercury. The diverticulum would tolerate considerable manipulation without leaking at the suture line. Clamping of the diverticulum close to the suture line, however, invariably resulted in hemorrhage at the junction of the diverticulum with the heart. In only one experiment was an attempt made to sew a diverticulum onto the aorta. The bleeding around the suture line was severe and this was the only animal, where the diverticulum had been used, in which death, as the result of hemorrhage, occurred.

In only one animal has there been any gross evidence of interference with the coronary artery supply to the myocardium following removal of the diverticulum. In this animal a large branch of the conornary artery was apparently injured during the suturing of the diverticulum or closure of the incision.

Manipulations with the finger within the ventricle resulted in marked arrhythmia if the finger was moved about rapidly or roughly, and in a marked fall in blood pressure if either the inflow or outflow tracts to the

ventricular chamber were partially or completely blocked (Fig. 3). The very rapid administration intravenously of a concentrated solution of norepinephine (Levophed) was given to two animals with a right ventricle diverticulum and one animal with a left ventricle diverticulum. Ventricular fibrillation followed immediately. The slower administration of this drug to a number of other animals resulted only in the expected pressor response and

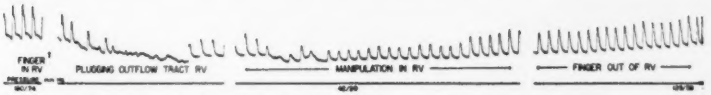

FIG. 3. Finger manipulation in right ventricle. Femoral artery pressures.

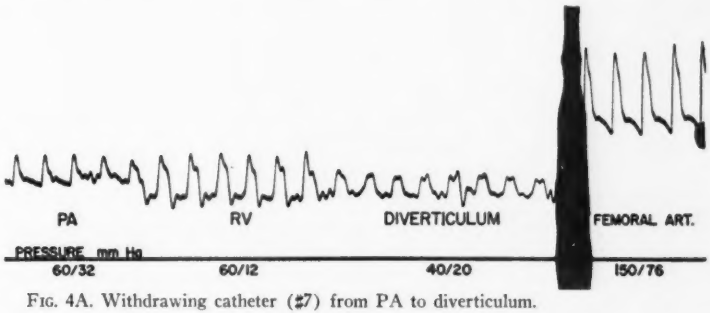

FIG. 4A. Withdrawing catheter (#7) from PA to diverticulum.

FIG. 4B. Withdrawing catheter (#7) from aorta to diverticulum.

tachycardia. The heart in cardiac arrest and ventricular fibrillation has been successfully resuscitated by massage and electric shock when the diverticulum was in place with only a minimal loss of blood from the suture line. Blood pressure recording through a No. 7 catheter revealed a lower systolic and pulse pressure in the diverticulum than in the ventricle (Fig. 4, A, B). Closure of the incision of the ventricle by the method described was usually efficiently accomplished with minimal blood loss and disturbance of cardiac function. The time that the communication was open between the diverticulum and the chamber of the ventricle varied from 10 to 50 minutes.

Recently we have made a diverticulum with three extensions as shown in Figure 5. The form is autoclaved prior to use and is used only once. We

have made this diverticulum by dipping, one or more times, a glass mold in liquid latex and then fixing and curing the rubber in the usual way. The over-all length of the diverticulum from the bead to the tip of the largest extension measures 60-65 millimeters, depending on how much rubber is used in rolling the bead. The bead measures about 2 millimeters in thickness. The length of the largest and middle extensions measures 20 millimeters, and the smallest extension, 10 millimeters. The internal diameter of the largest extension is 15 millimeters, the middle extension 10 millimeters, and the smallest extension 5 millimeters. The internal diameter of the diverticulum at the level of the bead is 30 millimeters in the long axis of the oval form and 15 millimeters in the transverse axis of the form. As in the diverticulum with two extensions, the bead is rolled over on itself once, prior to suturing to the myocardium.

Comment

The success of this technique for the digital exploration of the chambers of the ventricles, with minimal blood loss, is due in large part to anatomical and physiological factors: namely, the thick muscular wall of the ventricle and the contraction of this muscle during systole, which tends to close the incision during the phase of increased pressure within the ventricular chamber. This sphincter-like effect can best be appreciated when the tip of the finger is placed in the incision. Further, as the ventricle contracts it tends to pull the ring of the diverticulum close to the myocardium to which it is sewed. It is questionable if this technique can be applied with equal success to the failing heart with a dilated, relatively thin-walled ventricle.

It is suggested that this method of approach to the ventricular chambers of the heart might be considered when an attempt is made at the partial or complete correction of several intracardiac defects, as well as the removal of intracardiac foreign bodies and tumors. We believe that before this technique is used in the human for the correction of specific intracardiac defects or disease, a similar condition, as near to it as is possible, should be made and corrected in the experimental animal.

Acknowledgment

The author gratefully acknowledges the assistance of Dr. William E. Bloomer, Mr. Armand Negri, and Dr. Hayes H. Cluxton in this work.

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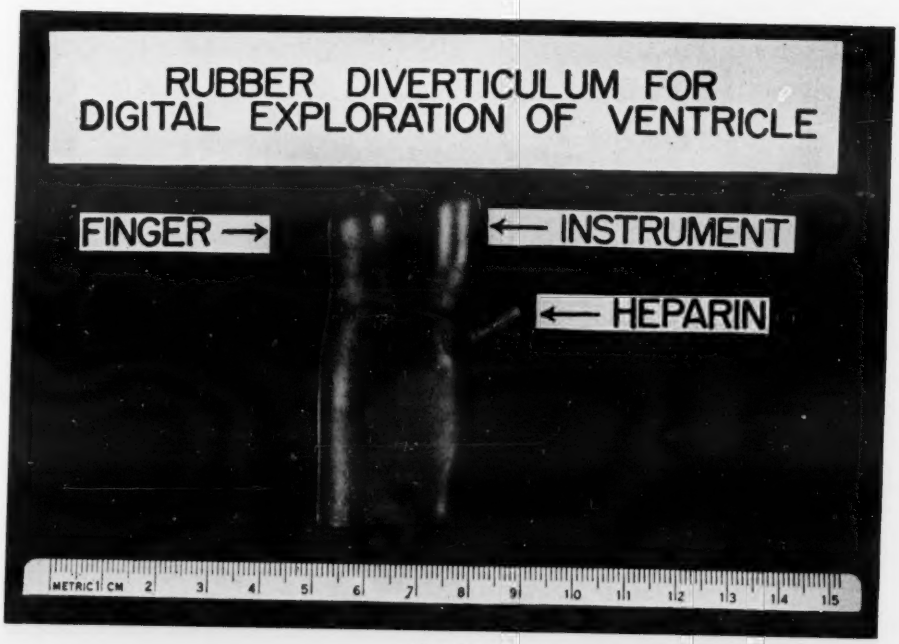
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FIG. 5

THE EFFECT OF LYSOZYME ON THE PRODUCTION OF TETANUS TOXIN. II. MOUSE M.L.D.*

JOSEPH L. STONE

It has previously been reported[1] that during the production of tetanus toxin in the Mueller Medium, the medium becomes dark and turbid in about 24 hours, followed by clearing in four or five days. When clearing occurs, maximum toxin production or release is obtained as measured by Lf units. The simultaneous occurrence of clearing and presence of maximum Lf value has been attributed to "autolysis" of the organisms with simultaneous release of toxin.[2] An attempt to demonstrate an increased yield in toxin production by artificial lysis of the organisms with lysozyme revealed no rise in Lf titer, although there was a marked decrease in time of flocculation due to the presence of lysozyme during the early stages of growth.[4]

The present report deals with the effect of lysozyme on tetanus cultures as measured by their toxicity in mice.

Materials and Methods

The medium used for the production of tetanus toxin was prepared according to the most recent Mueller formula, consisting chiefly of "N-Z case," veal infusion, glucose, and several salts, amino acids, and vitamins.[3] The culture of *Clostridium tetani* (Harvard strain) was planted from the frozen state into a fluid thioglycollate medium and grown for 24 hours prior to inoculation into the toxin production medium proposed by Mueller. The lysozyme was a crystalline product prepared from egg white. Dilutions of the enzyme were made in Sørensen's phosphate-buffer at pH 6.29.

M.L.D. determination. A sample of the culture for lysozyme treatment was removed from a regular production lot and divided into three portions of 1.9, 1.9, and 2.0 ml. To the two 1.9 ml. samples were added 0.1 ml. of a lysozyme solution (100 mg. per ml.) and 0.1 ml. of the phosphate buffer respectively. The three tubes now containing 2.0 ml. each were incubated at 35° C. for six hours. Dilutions of these samples were then made in peptone-saline solution for mouse injection. Three separate dilutions were prepared from each of the three samples on each day of growth for injection into mice. Two mice weighing 16-22 grams were each injected intramuscularly into the left thigh with 0.5 ml. of each dilution. The minimal lethal dose was calculated on the basis of the result in each mouse injected according to the method of Ipsen.[5]

Gram stains were prepared from specimens of lysozyme-culture mixture, buffer-culture mixture, and plain culture after the tubes had been incubated for four hours.

Results

When samples from early phases of growth (24, 48, and 72 hours) were treated with lysozyme (5 mg. per ml. final concentration), there was an initial flocculation, occurring in 10 to 15 minutes, followed by a marked

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clearing which was complete in about two hours. This phenomenon did not occur with the two control samples containing either buffer solution and culture or culture only. In addition, clearing was not as evident in the lysozyme tube with the four-, five-, and six-day growths when natural autolysis was occurring most rapidly or was in its final stages.

Stained preparations made during various stages of growth confirmed the clearing effect of lysozyme on the culture. Stains from one-, two-, and three-day samples that were incubated with lysozyme showed rare Gram-negative cells, many ghost cells, and much débris, whereas corresponding control

TABLE 1

COMPARATIVE MEAN LOGARITHMIC M.L.D. VALUES ON LYSOZYME-TREATED AND CONTROL CULTURES OF ONE- TO SIX-DAY GROWTH DURING THE PRODUCTION OF TETANUS TOXIN

Days of growth of culture	*Lysozyme plus culture* Experiment no.*				*Buffer plus culture* Experiment no.*				*Culture only* Experiment no.*			
	III	IV	VI	VII	III	IV	VI	VII	III	IV	VI	VII
1	3.40	4.15	...	<3.30	3.01	3.66	...	3.06	3.08	3.76	...	3.22
2	4.99	5.34	...	5.56	3.87	4.51	...	3.80	3.89	4.37	...	3.94
3	5.59	5.77	5.86	5.78	5.14	5.29	5.43	5.19	5.35	5.30	5.21	5.12
4	...	...	6.31	6.43	...	...	6.27	6.28	...	...	6.09	6.25
5	...	...	...	5.99	...	...	...	5.98	...	...	...	6.02
6	...	...	6.05	5.80	...	...	6.06	5.89	...	...	6.03	5.85

* Placed in incubator for six hours prior to dilution for M.L.D. determination.

samples contained large numbers of short and long rods typical of the Harvard strain of *Clostridium tetani*. In this respect, also, four-, five-, and six-day cultures were less visibly affected by lysozyme, since the enzyme-treated and control cultures were alike in revealing very few whole organisms. In the older cultures the organisms had apparently already undergone lysis prior to the addition of lysozyme.

The effect on toxicity of the treatment of tetanus cultures with lysozyme is shown in Table 1. The figures in this table represent the geometric mean of the M.L.D. values on six mice in most instances. It is evident from this table that only small amounts of toxin were present in the medium after 24 hours of growth, regardless of whether or not the culture was treated with lysozyme. There was, however, an increase in the amount of toxin present in the 24-hour culture as a result of lysozyme treatment in Experiments III and IV. In Experiment III, the logarithmic mean of the M.L.D. value of the lysozyme culture was 3.40, whereas the mean M.L.D. values of the buffer plus culture control, and the culture only were 3.01 and 3.08, respectively. These figures indicate a per cent increase in M.L.D. for

lysozyme-treated over the controls of 145 and 110 whereas there was only a 17% difference between the controls. For Experiment IV the mean differences were approximately the same with per cent rises in M.L.D. value of lysozyme-treated cultures over the controls of 210 and 144. The difference between the controls in Experiment IV was 27 per cent. Table 1 also reveals that the maximal effects of lysozyme in increasing the M.L.D. in mice was on two-day cultures. On the three-day culture there was a sharp drop in effectiveness of lysozyme. Four-, five-, and six-day growths revealed little or no effect due to lysozyme.

TABLE 2

DIFFERENCES BETWEEN MEAN M.L.D. VALUES OF LYSOZYME-TREATED TETANUS CULTURES AND THE CONTROL CULTURES

Days of growth	*Mean M.L.D. of:*		*Factor of difference in value between*	
	Lysozyme plus culture L	*Buffer-culture controls* B-C	*L and average of* B-C	B and C
1	8,320	2,560	3.31	1.0
2	226,000	14,200	15.9	1.2
3	575,700	184,350	3.1	1.1
4	2,355,000	1,703,000	1.4	1.2
5	980,000	999,500	1.0	1.1
6	874,000	939,000	1.1	1.1

The increase in M.L.D. value as a result of lysozyme treatment for the six days of growth in comparison with the difference in M.L.D. value of the controls (buffer plus culture and culture only) is summarized in Table 2. This table reveals that there is some rise in M.L.D. on the first three days of growth as a result of treatment with lysozyme, the most marked rise in M.L.D. being in the two-day culture with a maximal rise of 15.9 times, while the difference between the two controls was only 1.2. No significant difference can be noted in Table 2 in mouse M.L.D. between the lysozyme-treated and the control samples for the four-, five-, and six-day growths.

The results of lysozyme treatment on the four- to six-day growth as compared with the untreated cultures can also be seen in Table 1. The M.L.D. values in this table reveal no increases as a result of lysozyme activity on the culture at five and six days. There appears to be a slightly higher mean M.L.D. value for the lysozyme-treated culture in both Experiment VI and VII on the fourth day, but the significance of this difference might be considered doubtful.

Table 2 and Experiments VI and VII of Table 1 appear to indicate also that the maximal mouse M.L.D. value is reached after four days of incuba-

tion by this method of toxin production. This peak is followed by a slight decrease in M.L.D. on the five- and six-day-old cultures. Evidently when the peak M.L.D. value is reached, lysozyme treatment thereafter does not increase the toxicity of cultures to mice.

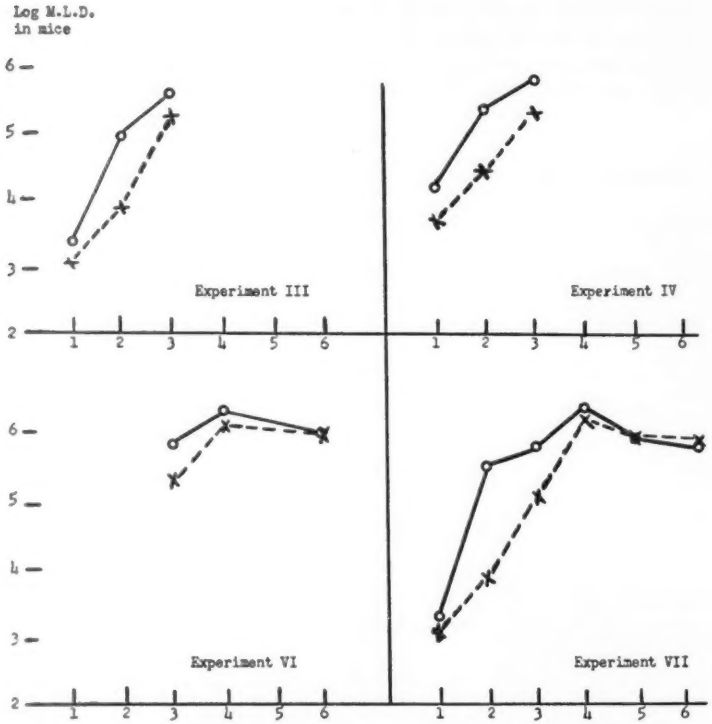

FIG. 1. The log of the M.L.D. in mice for each day of incubation comparing samples of cultures treated with lysozyme (circles and solid lines) and the average results of the controls (x's and dotted lines) for each of four experiments.

Figure 1 illustrates graphically the results of Tables 1 and 2. For each day of growth the M.L.D. value rises in each experiment to a peak at four days, and following the three-day growth, the lysozyme curve and the curve representing the average of the controls approach each other, indicating decreasing difference in M.L.D. value for lysozyme versus controls.

In order to make more certain that the lysozyme activity was directed on the cells and not on some material in solution, supernatants of cultures centrifuged for 30 minutes were treated in the same manner as whole cultures after each of the first three days of growth. On none of these three

days was there any evidence of toxin present in either lysozyme-treated or untreated supernatant fluids at the lowest dilutions tested which were 1-100, 1-2000, and 1-50,000 for one, two, and three days' growth, respectively. Untreated, whole culture controls for these three days showed mean M.L.D. values of 2,200 for the one-day growth, 12,000 for the two-day growth, and 288,000 for the three-day growth.

In order to rule out the possible toxicity of lysozyme itself as an influential factor in causing the increased M.L.D., lysozyme alone in 0.2 mg. quantities was injected into each of two mice. This represents nearly ten times the greatest amount of lysozyme introduced into mice in the lysozyme-toxin experiments. The mice were observed for seven days and showed no signs of toxicity at any time during this period.

Discussion

As a method of further evaluating the Mueller hypothesis that tetanus toxin was liberated into the medium at the time the organisms underwent autolysis on about the fifth day of growth, lysozyme was used to lyse these cultures artificially in the early (first three days) stages of growth. As a result of induced lysis of the tetanus bacilli, M.L.D. values in mice were increased as much as 16 times on two-day cultures, at which time the maximum effect of lysozyme occurred.

Although there was a marked enhancement of the toxicity to mice of tetanus broth cultures as a result of treatment with lysozyme on the first three days of growth, there were no instances in which maximal yields of toxin were obtained during these three days despite apparently complete lysis. This can be explained by the possibility that the organisms had not as yet manufactured maximal amounts of toxin even after three days of growth and that the only effect of lysozyme is to liberate all the existing toxin from the cells.

It seems to be evident that the greatest yield of toxin as measured by mouse M.L.D. can be obtained after four days of incubation. At this time the culture shows its earliest gross and microscopic evidence of being autolyzed and is similar in appearance to younger cultures that have been incubated with lysozyme for four hours. Also at this time, lysozyme begins to show a lack of activity on the culture in respect to increasing the M.L.D. value in mice. After four days of growth, the mouse M.L.D. begins to drop so that it is lower after five days and still lower after six days. This decreasing toxicity undoubtedly represents natural self-detoxification of the tetanus toxin, a phenomenon which is commonly known and accepted. During the last two days of growth lysozyme has no effect on toxicity.

Supernatants from centrifuged early cultures (one, two, and three days) that were treated with lysozyme resembled the untreated supernatants in showing no signs of toxicity for mice. Corresponding whole cultures showed normal M.L.D. values during these days. This once more indicates that the

toxin is associated with the cell and is not liberated until the cell ruptures. It does not, however, necessarily follow that the toxin is present or remains within the cell membrane, since it may possibly be adsorbed to the surface of the cell, remaining there until the cell is lysed.

Summary

1. Samples from tetanus cultures were treated with lysozyme, to produce lysis, during each day of six days of growth.

2. Lysozyme produced lysis of the organisms during the first three days of growth, but had no effect during the last three days since autolysis had already occurred.

3. The toxicity of the cultures, measured as mouse M.L.D., was markedly enhanced by lysozyme treatment of the first three days' growth, but there was no effect on cultures four to six days old.

4. The maximal M.L.D. value in mice was obtained after four days of growth regardless of whether or not the cultures were treated with lysozyme.

5. Supernatant fluids, both untreated and lysozyme-treated, from centrifuged cultures, one to three days old, showed little or no toxicity for mice.

Acknowledgments

I wish to express my gratitude to Dr. Johannes Ipsen and Mr. Leo Levine for helpful suggestions in the preparation of this paper, particularly regarding some of the statistical expressions.

Acknowledgment is made to Mrs. Sally Ostrowsky for technical assistance.

I am indebted to Armour and Company (through Arlan G. Roberts, Research Division) for the generous supply of lysozyme used in this study.

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A CLINICAL AND EXPERIMENTAL DETERMINATION OF PRESSURE WITHIN THE CAROTID ARTERIES*

WILLIAM J. GERMAN AND SAMUEL P. W. BLACK

During the past fifteen years a new field of neurological surgery has been developed; this might be called intracranial vascular surgery. One of the most challenging problems in this field is the treatment of intracranial aneurysms. About 75% of these aneurysms[4] lie in or near the anterior half of the circle of Willis, receiving their major blood supply from the internal carotid arteries. Most of these anteriorly placed aneurysms can be localized precisely by angiography. We believe that those arising from the internal carotid arteries can be very favorably influenced by carotid ligation in the neck.[8] The purpose of the present paper is to report on our studies of intracarotid pressures under various circumstances and to suggest a preliminary correlation of these data with probable forces acting upon intracranial aneurysms.

METHODS

Intra-arterial pressures were measured by means of an inductance-type gauge, amplified through a vacuum tube system, and recorded on a photographic galvanometer. A method similar to this has been employed clinically by others[1, 2, 6] in their studies upon intravascular pressure throughout the ramifications of the internal carotid artery. Intra-arterial pressure was applied to the gauge through a #18 or #19 flexible needle. All determinations were made with the carotid arteries exposed.

RESULTS

Figure 1 shows intra-arterial pressure recordings from the common carotid artery. In section A there is no obstruction to the blood flow and the blood pressure† averages 124/54, mean pressure‡ 89, pulse pressure 70. When the common carotid was occluded, B, proximal to the pressure recording site, the B.P. fell to 43/30, mean pressure 36, and pulse pressure 13. These pressures were contributed by the external and internal carotid

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† All pressures are cited in millimeters of Hg.

‡ Mean pressure was determined by averaging the systolic and diastolic pressures. This might be termed the arithmetical mean pressure in contradistinction to the more accurate method of mean pressure determination wherein the blood pressure curve is integrated. Of the two methods the former is the more practical because it is available immediately for clinical decisions. There is some difference in the results obtained by the two methods. For example, in Figure 1A the arithmetical mean pressure is 89.0, while by the integrative method it is 80.0; in Figure 1C it is 36.5, arithmetically compared to 34.0. In Figure 4A the arithmetical mean pressure is 122.5, while by the integrative method it is 116.8; in Figure 4C it is 79.5, arithmetically compared to 80.5.

arteries and represent collateral and reflux pressures respectively. In section C both the common and external carotid arteries were occluded with reduction of B.P. to 31/29, mean pressure 30, pulse pressure 2. Immediately after this (not shown in figure) all occlusions were removed and the intra-arterial pressures returned to the original levels. It is apparent from these results that a very considerable reduction of peak-pressure (65%), mean

FIG. 1A. Intra-arterial pressure recording from common carotid.
FIG. 1B. Collateral and reflux pressure from external and internal carotid arteries respectively.
FIG. 1C. Reflux pressure from internal carotid; note very low pulse pressure.

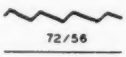

FIG. 2. Intra-arterial pressure recording from common carotid one year after proximal ligation; compare with Figure 1A.

pressure (60%), and pulse pressure (81%) occurred distal to the common carotid occlusion. Combined common and external carotid occlusion, which is the same as internal carotid occlusion, gave even more striking reductions: peak-pressure 75%, mean pressure 66%, pulse pressure 97%.

Figure 2 illustrates that low pressures may be maintained for a significant time after proximal ligation. This patient had the common carotid ligated

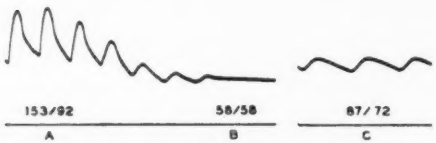

FIG. 4A. Intra-arterial pressure recording from common carotid.
FIG. 4B. Effect of proximal occlusion of common carotid; note disappearance of pulse pressure.
FIG. 4C. Effect of partial ligation of common carotid.

one year previously and these pressure recordings were obtained from the external carotid, near its origin. Average pressures at this site were B.P. 72/56, mean pressure 64, pulse pressure 16. Simultaneous sphygmomanometer pressures in the brachial artery were B.P. 130/90, mean pressure 110, pulse pressure 40. Thus the intra-arterial pressures distal to the common carotid ligature were reduced in relation to systemic pressures as follows: peak-pressure 45%, mean pressure 42%, pulse pressure 60%. A considerable reduction in the demonstrable size of this patient's internal carotid aneurysm occurred during the year following common carotid ligation (Fig. 3).

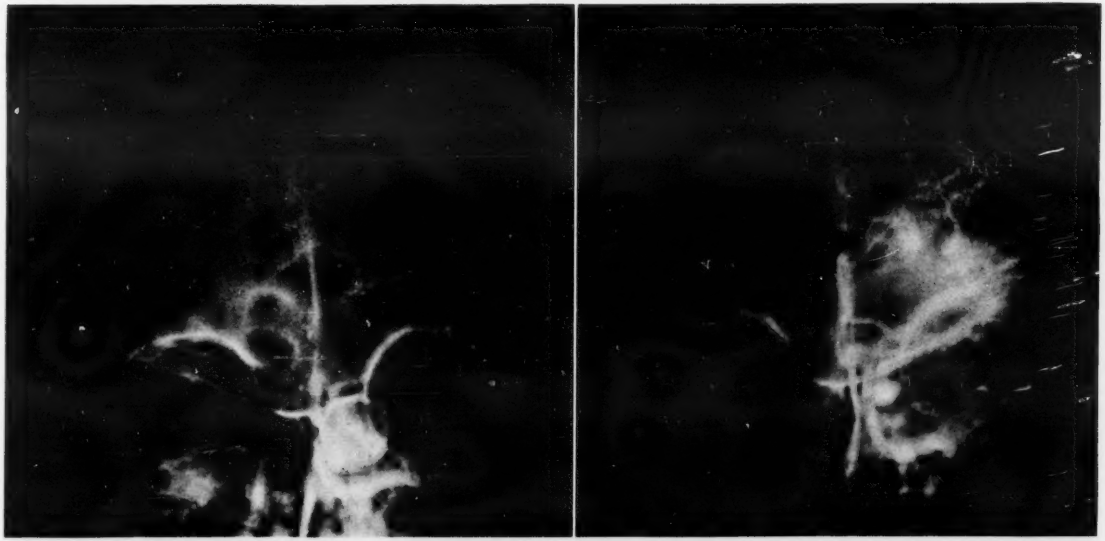

FIG. 3. Arteriograms showing internal carotid aneurysm before left common carotid ligation and one year later; note marked reduction in size of aneurysmal cavity.

On a few occasions it has appeared inadvisable to perform a complete ligation of the common carotid artery, because of probable consequent serious cerebral vascular deficit. In these cases, partial ligation has been done, using several in-folding mattress sutures into the muscularis of the common carotid.[5] Pressure studies in such a case are illustrated in Figure 4. Pressures in the common carotid immediately preceding trial occlusion, A, were: B.P. 153/92, mean pressure 122, pulse pressure 61. As the common carotid was occluded, B, the pressures distal to the occlusion fell to B.P. 58/58, mean pressure 58, pulse pressure 0. The pressure reductions were: peak-pressure 62%, mean pressure 52%, pulse pressure 100%. After partial ligation of the common carotid, C, the pressures were: B.P. 87/72,

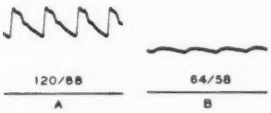

FIG. 5A. Common carotid pressure recording 26 days after proximal partial ligation; compare with Figure 4C.

FIG. 5B. Effect of proximal complete occlusion; note low pulse pressure.

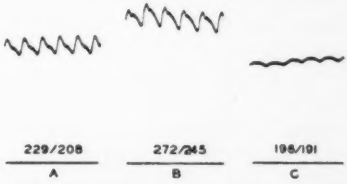

FIG. 6. Intra-arterial pressure recording from common carotid of a dog.

A. Side-pressure.
B. End-pressure; note change in wave form from A.
C. Collateral-reflux pressure; note low pulse pressure.

mean pressure 80, pulse pressure 15. The pressure reductions were: peak-pressure 43%, mean pressure 34%, pulse pressure 75%.

In one instance pressure studies were made 26 days after partial ligation of the common carotid, Figure 5. Pressures above the partial ligation, A, were: B.P. 120/88, mean pressure 104, pulse pressure 32. Complete occlusion of the common carotid, B, reduced the pressures to: B.P. 64/58, mean pressure 61, pulse pressure 6. The pressure reductions were: peak-pressure 47%, mean pressure 41%, pulse pressure 61%.

Recently, studies have been initiated in the experimental laboratory for the purpose of determining various types of pressures to which an artery may be subjected. The common carotid artery in the dog gives off a small thyroid branch, approximately at right angles to the parent trunk. Cannulization of this branch permits determination of side-pressure within the major artery. By temporary occlusion of the common carotid cephalad or cardiad to this branch, end-pressure and collateral-reflux pressure, respectively, may be determined. Figure 6 illustrates the relationships of these three different pressures. In section A there was no obstruction to blood flow in the common carotid and the side-pressures were: B.P. 229/208, mean pressure 218, pulse pressure 21. When the common carotid was

occluded cephalad to the site of pressure measurement, B, the end-pressures were: B.P. 272/245, mean pressure 258, pulse pressure 27. With cardiad occlusion of the common carotid, C, the collateral-reflux pressures were: B.P. 196/191, mean pressure 193, pulse pressure 5.

Discussion

The correlation of these data with probable forces acting upon intracranial aneurysms requires brief consideration of certain factors of theoretical hydraulics and cerebral circulation. The stresses operating to produce strains within an aneurysm include: total hydrostatic pressure in the parent artery, pulsatile flow, turbulence, and jet action. Total hydrostatic pressure is equal to pressure head plus velocity head. Under normal conditions in the circulatory system, with its relatively high pressure and low velocity, the velocity head is of little importance. The average strain upon an aneurysm from hydrostatic pressure is therefore proportional to the mean arterial pressure in the parent artery. In the cases illustrated in Figures 1 and 2, mean pressures were reduced 60% and 42% immediately and one year respectively after common carotid occlusion.

The second stress factor, pulsatile flow, may be considered as having a waterhammer effect within the aneurysm. The cardiac systolic thrust creates a pressure wave which travels at a much faster rate than the blood, at least 350 cm./sec. A pressure head of but 10 mm. Hg., for example, riding the blood stream at this speed, is capable of a thrust of about five times that amount against the walls of a closed sac such as an aneurysm. While this pressure wave is not identical with the pulse pressure, the two are probably influenced in a proportional manner by carotid ligation. Referring again to the cases illustrated in Figures 1 and 2, pulse pressures were reduced 81% and 60% immediately and one year, respectively, after common carotid occlusion.

We shall omit discussion of the factor of turbulence. Jet action, however, may be one of the most important elements in aneurysmal rupture. The theoretical energy of a jet varies with the *cube* of the velocity. Blood issuing through an aneurysmal neck 1 mm. in diameter, at the side of the parent artery carrying a pressure of 100 mm. Hg., would have a theoretical velocity of about 500 cm./sec. With the aneurysm intact, jet action is greatly dampened by the blood in the sac. Pulsatile changes in the parent artery, however, would permit an ebb and flow of low intensity jet action. There are two components to the force of a jet, the impulse and the reaction, each of which varies as the square of the velocity, the former tending to drive the aneurysm away from the artery, the latter to tear the artery from the aneurysm. When a leak in the aneurysm occurs, full jet action may be expected, the dynamic pressure caused by the jet being *double* the hydrostatic pressure within the parent vessel. From the above considerations it may be inferred that jet effect in the intact aneurysm would be proportional

to pulse pressure, while jet action from a leaking aneurysm would be proportional to mean pressure. Carotid ligation would therefore tend to reduce the jet action stress by the same amounts as previously estimated for pulsatile flow and hydrostatic pressure.

Finally, brief mention should be made of certain factors related to intra-arterial pressure measurements. Artifacts due to inertia, dampening, friction, and fluid flow are overcome to a large extent in the modern apparatus. The introduction of a pressure-recording mechanism into the lumen of an artery decreases the available area for the flow of blood in proportion to the relative size of the needle to the artery. If the needle is directed cardiad, the measurement is a mixture of "normal" pressure, end-pressure, and velocity head. If the needle is directed cephalad, the velocity head becomes negative. Pressure measurements distal to an occlusion of the common carotid have been referred to previously as collateral-reflux pressures. Perhaps future analyses of pressure wave forms will permit differentiation of the external carotid collateral pressure from that due to internal carotid reflux flow.

Summary and Conclusions

The empirical treatment of internal carotid aneurysms by proximal ligation has been evaluated by means of intra-arterial pressure studies. A preliminary correlation of these data with probable forces acting upon intracranial aneurysms has been made. It is concluded that the treatment of internal carotid aneurysms by proximal ligation has a sound basis, both in respect to theoretical hydraulics and to the physiology of cerebral circulation.

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JOH. FRIEDRICH MIESCHER AND THE CONTRIBUTION OF BASLE PHYSICIANS TO THE BIOLOGY OF THE NINETEENTH CENTURY*

HEINRICH BUESS**

Let me first of all tell you how glad I am to have spent a few weeks of my time in the United States in the magnificent Historical Library of Yale University. And let me express my deepest thanks to Dr. John F. Fulton for the suggestions he has given me from his wide knowledge and for his advice concerning the books of the Historical Library, which contains such a wealth of precious items of all kinds. Although in Basle we also have an excellent library, I must confess that I envy the students and doctors here the facilities which are always at their disposal in the treasures collected by Doctors Cushing, Klebs, and Fulton himself.

The first two scholars—Dr. Cushing and Dr. Klebs—had many contacts with Switzerland, since Harvey Cushing (1869-1939) as a young surgeon worked during the winter of 1900-1901 at the clinic of Theodor Kocher (1841-1917) and enthusiastically reported his observations (in word and in drawings) on his visit in Berne and also in Lausanne and Geneva, and many of you know that Cushing regarded Kocher as one of the teachers whose precepts accompanied him to the Johns Hopkins Hospital and even after. The names of Edwin and Arnold Klebs are also closely connected with the development of medical sciences in our country, for in the field of bacteriology the elder Klebs' (1834-1913) work during the time he spent in Switzerland led to his dramatic discoveries, and his son (1870-1943), the learned doctor of Nyon on Lake Geneva, became one of the founders of our Swiss Association of the History of Medicine almost thirty years ago. It has been very pleasant for me to see from the several invitations I have received how lively the interest in our small country is among different people of your great land, here at Yale and at other places.

Hence, it may interest you to hear something of the contribution of Basle physicians to the history of biology in the nineteenth century. Especially worthy of attention is a physiologist whose name is known only to a few in English-American medical literature, despite the fact that he was one of the outstanding investigators in biochemistry in its early days. Before touching upon the life and work of Dr. Fritz Miescher (1844-1895), however, it will be useful to learn something of his historical background, that is, something of the past and present of the town and the University of Basle up to his time.

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* From the Department of the History of Medicine, Yale University School of Medicine. Read in the Historical Library, February 14, 1952.
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How did our city acquire its important rôle in the development of medicine in Switzerland? Last year we had the pleasure of celebrating the 450th anniversary of the entrance of Basle into the Swiss confederation (founded in 1291). But at that time, that is, in 1501, the town in the northeastern corner of our land had already become well known as a center of commerce; also, since the beginning of the Renaissance, Basle had attracted many printers, especially from southern Germany. If you will look in some of the old books in this library, you will find that many of them bear the name of Basle on the title-page. Undoubtedly, the establishment of a university within its walls in 1460 contributed much to the formation of a humanistic center which became even more famous with the publication of the great work of Vesalius in 1543 from the press of Oporinus. One also remembers how happily Paracelsus delivered his lectures in the vernacular among the citizens of Basle.

It might be mentioned that the University developed quickly during the Reformation and anti-Reformation. It was the time of so-called liberalism, or more precisely, the restoration during the 1830's, when the University of Basle went through one of its most critical stages. I cannot go into details here, but I am sure that at Yale University, as well as at Basle, there have been, from time to time, men of wisdom conscious of responsibility who have helped to further the growth of their beloved institutions. So Basle established its medical instruction on a new practical basis, and starting from this point, we see the members of three Swiss families who, during two generations, were responsible for new developments in biological research within our city. After the first advances in the sixteenth century, we encounter another surge of growth in the sixties and seventies of the nineteenth century. At that time Basle was in strong competition with the newly founded universities of Zürich and Bern. In these towns there were among the newly appointed professors many Germans (such as Schoenlein, Billroth, Ludwig, etc.) who, by their energy, did much to stimulate medical research.

How much depends on the leading personality at a university is shown by the fact that one single scholar from an old family, Peter Merian (1793-1883), who was at that time Chancellor of the University, had great influence and possessed a fine understanding of the qualifications of the young doctors to be selected for new jobs. To one of them, a man of twenty-six, he said: "We throw you into the water; you might now look how you will swim." This appointment of Wilhelm His, the anatomist (1831-1904), was to be one of the most fortunate ever made in the history of our University. There were fathers and sons of three families, namely, Miescher, Rütimeyer, and His, who contributed most to the reputation of the Basle school in the biological field. Sterling Library of Yale University possesses many of the writings of each of these physicians.

Friedrich Miescher, the elder (1811-1887), and Wilhelm His bring us into contact with the most outstanding German biologist of the first half of the nineteenth century, namely, Johannes Müller (1801-1858), the founder of the German school of anatomy and physiology. It was Müller, with his earnest mien and strange charms, who evoked from His the enthusiastic words: "Already the first lectures have worked upon me like a revelation, and the longer I stay the more I learn what it means to come under the influence of so mighty a personality." It was Müller who suggested that the elder Miescher study the different processes underlying inflammation of bones, and Müller himself wrote the foreword to the treatise. It is interesting to observe how one single feature of character and mind had power over the scientific activity of both Mieschers, with the result that they were extremely reluctant to bring forth the findings of their investigations—a fact which makes it difficult for both biographer and historian to reach a well-defined judgment. Thus we see that, despite slight literary productivity, the elder Miescher played a significant rôle in the development of the younger generation, particularly in the development of his son, the biochemist, and of His, the anatomist.

Before returning to the representatives of biology in Basle, let me call your attention to a feature common to most Swiss scientific activity and one which confers upon it a specific aspect, that is, the orientation in the direction of German as well as French culture. It may be observed that this position between these two nations protects the Swiss historian from a chauvinism which is sometimes so symptomatic of scholars, especially in the field of biography. By the end of the eighteenth century France had produced an extensive literature on comparative anatomy. That the city of Basle in its Museum of Natural History has one of the best collections in this area of science is due to the varied interests of one of the University teachers.

This man was Ludwig Rütimeyer (1825-1895), of Bern Canton, an originator of the prealpine zone, who had brought, apparently from his studies in Paris, a definite plan for further research. It might have been his former studies in theology and philosophy which led him to trace the forms of the recent animals back to their earliest stages of development in prehistoric time. His inclination toward practical medicine was slight—the same disinclination probably appeared in other outstanding Swiss physicians at that time—and it is reported by Rütimeyer himself that, in a kind of flight, he left a district hospital to concentrate his endeavors on the "Jardin des Plantes" in Paris. In London, he was an admirer of the genius of the great Richard Owen (1804-1892) at the British Museum. With regard to his contributions to the history of biology it may be said that he introduced the method of "historical paleontology" into many new fields of natural science. Through his observation of the particularities of his subject, together with his wonderful capacity for philosophical abstraction, he recog-

nized in the crania, jaws, and teeth of ancient animals "a direct history which should continue to the present time."

Without doubt, comparative anatomy forms the basis of Rütimeyer's investigations. The historical approach was suggested by the discovery of the lake-dwellings on the lake of Zürich; from these he could follow not only the development of animals back through thousands of years, but he was also able to compare the forms of ancient skulls, etc. within different parts of the globe and thus found important kindred relations to the fauna of North America. This method of comparison spread over from France and England to Germany, and it is well known that the master of the German school of biologists, Johannes Müller, in his later years, became more and more interested in comparative anatomy. The next step in this kind of research, after the cytology was established by a pupil of Müller, carried the embryological pattern in a new direction, namely, to the observation of the formation of cells and their development.

The fact that Wilhelm His, a brother-in-law of the elder Miescher, concentrated his studies more and more on developmental physiology has a bearing too on the dominant rôle of Müller as the founder of a widespread school. Since His helped to obtain recognition for the works of the younger Miescher in biochemistry, it might be well to survey his life and work. His was born in Basle in 1831; his father belonged to the highest social group of Basle aristocracy which, at that time, was more differentiated than now. Here we have a point of contact with an important feature in the history of Basle, namely, the commercial side of its development. The industry of silk-weaving gained an increasing influence and a strong position. His's ancestors were outstanding men of commerce, industry, and politics. One of them actively participated in the revolution in Switzerland which followed the one in France. After a thorough education and graduation from a lycée in which the classical tradition was strong, His, like Rütimeyer, came under the influence of an inspiring scientist in Bern, the geologist Bernhard Studer (1794-1887). The teaching of both young doctors—who received appointments at the University of Basle at about the same time, Rütimeyer in zoology, His in anatomy and physiology—may have reflected the enthusiasm of their teacher. His spent a certain period of his medical studies in Berlin, and it was here that Robert Remak (1815-1865), one of the founders of cytological embryology, directed his interests decidedly to this new area. Then, after a term in Würzburg, where he was attracted by the rising star of the young Rudolf Virchow (1821-1902), His was graduated from Basle, and for the next fifteen years taught there and built up a foundation of research which developed rapidly under his successors. Among the latter may be mentioned the German-American, Hanson Kelly Corning (born 1861), who died only last year in New York after having worked a long time in Basle. His textbook of topographical anatomy appeared in at least thirteen editions and was a basic work in surgical practice.

From 1872 until his resignation, His was Professor of Anatomy in Leipzig, and the newly established institute became a focal point for research in anatomy and embryology. Not only was he an excellent teacher (who supplemented his lectures with magnificent drawings on the blackboard), but he also was the originator of new methods of demonstration in topographical anatomy and embryology, among which are his famous plastic models of the bowels and of the development of the fetus.

What is the most important contribution of His to medical biology? I believe it lies in his macroscopical and microscopical studies on the formation of the nervous system. Since these studies are brilliantly published in many well-known treatises, it may be sufficient to mention only the most important of his neurological results. With regard to the work of his nephew, the younger Miescher, to whom we shall turn, it seems to be significant that His started his own investigations so consistently from the first cellular stages of the nervous system and that, principally, he always chose various kinds of animals for them. This foundation in cytology and comparative anatomy was to be the same for Miescher, as we will see. Even now, we can see a new connection with the later pattern of biochemistry.

After his first steps in the direction of embryology in Basle, the chief work of His was done in Leipzig. The key idea, like that of the process of folding which His took from geology, played an important rôle in the explanation of the complicated development of the human brain. The forces of folding are not the same in all directions, and poles of the upper part of the nervous cord influence the formation of the different contortions and trenches. As you see, here were already indications of developmental mechanics, a new morphological science growing up at that time. This basic research enabled His to make standard models to demonstrate these relations. For information concerning his joint embryological and histological research, I suggest reference to the published volumes and illustrations.

Only a few more words need be added. As a young privatdocent, in his first public lecture in Basle, His had already dealt with the development of the nervous cell; then he proved the formation of the spinal ganglia from the intermediate column of the spinal cord; finally, in the summer of 1886, he described the growing out of the nerve fibres from the so-called "neuroblasts," a certain group of cells in the spinal cord. This histo-embryological proof was important in the understanding of the nervous function and formed the morphogenetic basis for the neurone theory.

So far we have regarded His as a man of laboratory research, but, in an historical lecture particularly, we should complete the picture of our Basle doctor by mentioning his great interest in the history of science and old medical literature. Although these endeavors started from a pragmatical standpoint, His became interested in ancient biology through his own research, with the result that he wrote an excellent paper on the theories of generation up to the time of Albrecht Haller (1708-1777). This treatise is

not widely known, and is omitted, for instance, in the work of Joseph Needham (1935). In his later years this outstanding anatomist also brought forth contributions to the local history of his native town, and, what is even more important to us, collected all available writings of his nephew, Friedrich Miescher-Rüsch (1844-1895). One might say that it was this kinship that led His to this work of editing. However, in the history of biochemistry Miescher merits a high position. Thus, we must be grateful to Wilhelm His for undertaking such work for his young friend. Above all it is his letters written to His which best reveal to us the fine character of a modest scholar.

Thus, after having considered the characteristics of his scientific nature, let us try to give some idea of the personality of Miescher. I am sorry to have to omit here a new biographical paper of Miescher the younger, which was published by some of our experts in Basle in 1944 at the celebration of his 100th anniversary. During most of his youth Miescher lived in Basle, where his already renowned father taught pathology at the University and practised as a gynecologist. That was the time when the system of full-time professorships was being slowly introduced in our country. Hence, it is understandable that Miescher encouraged his son to get a practical education, despite the special fitness for laboratory work which he had revealed at an early age.

Since 1828, Christian Friedrich Schönbein (1799-1868), the discoverer of ozone and gun-cotton, had taught chemistry in Basle, and the postulate which he expressed in 1863 may have been somehow significant for his kind of instruction. In the Reports of the Bavarian Academy of Sciences he wrote: "The results of the experiments that we carry out with organic substances in our laboratories often are able to throw some light on the chemical processes as they take place in the living organism; meanwhile, it seems to me that, as a rule, the manner by which the chemist deals with material, compared with the natural environment of animals and plants, is so violent that until now only in a few cases has the chemistry of the laboratory allowed conclusions concerning that of living nature. . . ." In addition to this theoretical reflection, it was perhaps the freshness of the instruction and research of Schönbein which inspired Miescher to his chemical studies. The large library of his father and the atmosphere of culture under the paternal roof brought our student, at an early age, in contact with thought of the middle of the last century, and he may have recognized the promising future of German natural science which, at that time, was just beginning to move its wings. In the higher grades of school Miescher was always the top boy of his class and at the age of seventeen he entered medical school, where he also ranked highest and where, despite his natural modesty, he held an official position in one of the largest Swiss student organizations. One term he spent at Göttingen, where he worked in the laboratory of Friedrich Wöhler (1800-1882), who became famous for the

first synthesis of urea from inorganic substances (1828). Wöhler had an excellent background in chemical research and was one of the most successful pupils of J. Berzelius (1779-1848), the Swedish master of chemistry. A long sickness of typhoid fever not only delayed Miescher's graduation, but also injured his hearing, a factor which may have contributed to his later isolation in his institute.

It was Miescher's uncle, His, who suggested advanced studies in a special area of biochemistry, namely, the study of chemical composition of the different tissues. Before dealing with the respective studies of Miescher in this field, let us follow, through his letters, his impressions of the two places where he came in contact with physiology. His first teacher was one of the pupils of Virchow, Felix Hoppe-Seyler (1825-1895), who, after being an assistant in pathological chemistry in Berlin, had been appointed as a professor of applied chemistry in Tübingen. Here he established his laboratory from which came the discoveries that founded a new science in Germany. In these hazardous surroundings Miescher may have contracted the lung disease which, despite the famous climate of the Swiss mountains, took him away so prematurely. This is very likely, since the young doctor seems to have worked without any concern for his health. After remaining a year and a half in this damp laboratory, Miescher went to the most outstanding center of physiological research in Germany, namely, to Leipzig, where Carl Ludwig (1816-1895), also a pupil of Müller, had surrounded himself with men of all nations. The letters of Miescher, who did not care for the experimental technique of Ludwig and his school, although he undertook a study of the functions of the spinal cord, vividly illustrate life at that great institute. I have tried to translate some of the passages, in no particular chronological order and without comment. In a letter addressed to Hoppe-Seyler he reports: "The Leipzig Institute was in this year formally a sort of international scientific purse: Italians, Frenchmen, Swedes, Norwegians, Russians, Americans, Mohammedans were crowded around the experimentation tables. . . . You can see that the most important schools of physiology were represented." Miescher continues: "Every one brings something with him, and even to my humble self it was permitted to give wise counsels in matters of hemoglobin. . . . By the way, all really difficult operations . . . have been done until now by the technician of the Institute, Salvenmoser, who has enormous knowledge in it . . . and without whom I could not imagine the Institute. . . ." In another, more intimate, letter to his parents, dated 30 November 1869, he writes: "Already I have done experiments together with the American, Bowditsch (*sic*)." He was one of the two men Miescher quotes by name. Undoubtedly he meant Henry Pickering Bowditch (1840-1911), who had earned laurels a few years before as a major in the cavalry in the Civil War, and who had recently been graduated from Harvard Medical School. It is known that under the direction of "the old master," as F. H. Garrison called Ludwig, Bowditch took the first

successful steps in his brilliant career as a leader of physiology in the United States. It is interesting that Miescher named "a certain Macallum of Toronto" (28 July 1893) who, a long time before, had found iron in the nucleus of the cells.

To return to Miescher, I would recommend that you read his most interesting letters, especially those concerning the time spent in Leipzig. I wish to add one more passage here: "I get more and more the conviction that in the many studies on blood gases the ideas are of Ludwig and the technical work, as far as it requires dexterity, is the merit of the servant Salvenmoser. . . . The respective Russian or Dutchman stood nearby, held perhaps the sponge or the towel, scarcely knew what was happening, at least on the train of thought; a few numbers dictated in his note-book and afterwards many visitors were much surprised to see that their names were printed in a wonderful paper."

I have quoted here in part these amusing reports of the young Swiss in order to show you the outstanding personality of Ludwig. In 1872, a year after publishing his first treatise on pus cells, the twenty-eight-year-old Miescher was appointed as professor of physiology at Basle; until that time anatomy and physiology had been taught together. After this Miescher carried for many years the heavy burden of instruction. Even six years later he complains to a colleague: "Now I sigh under twelve hours of weekly lectures, in addition to the practical courses and, being without an assistant, I come only now and then to my senses." He felt he should prepare his lectures so thoroughly that his research suffered for a long time; but in 1885 he got an institute of his own in the so-called Vesalianum (which was constructed a good deal according to Miescher's projects) and better equipment, which alleviated his task. Let me only mention here Miescher's contribution to dietetics in practical relation.

More and more he was becoming a prisoner of his disease and he had to undergo cures in the mountains; he was now aware that his scientific work would be cut short, as we can see from the following phrases: "But I should have fifty hands instead of two. . . . If I had as much time as material I would advance very fast. . . . I feel like a man in a debtors' prison. As long as I have not paid my old debts I cannot go on to new tasks. . . . Only when I read here and there an immature fragment of that which I have collected, printed by another author, do I recognize what could have been done with my material." Here we meet what I have already mentioned, namely, the extreme reluctance of Miescher to publish his results. In addition to his illness, this modesty has meant that a great part of his life work has remained unknown, and if His had not brought forth many letters, and if the well-known pharmacologist, Oswald Schmiedeberg (1838-1921), had not completed one of the last treatises, our picture of the scientific position of the Basle physiologist would be even less complete. Miescher felt this

deficiency keenly. In two letters to his uncle he had set down a full program of intended publications, but in another letter written to his colleague, Rudolf Böhm (who was born the same year as Miescher and died in 1926), he groans: "I shall never have the conscience which is needed for the happiness of a man, that I fulfill completely and in a harmonious manner a task given to me . . . and the main basic frame of my life always will be the unpleasant feeling of a man who has lost the bottom of his braces." So we understand quite well when one of his pupils compared Miescher with "a boat loaded with precious treasures which is sinking while entering the port." Miescher died in Davos, a health resort in our mountains, on 26 August 1895. Is it not the irony of fate that he should end his life in a climate toward whose scientific exploration he had contributed so much?

What are, then, the merits of Miescher in medical biology? To appreciate them we should give the outlines of the development particularly of vegetative physiology. Let us start with Miescher's teacher, Hoppe-Seyler, whom we called the founder of physiological chemistry in Germany. He dealt extensively with the chemical compounds of the blood and, together with the Frenchman, Georges Hayem (1841-1933), laid the basis of modern clinical hematology in relation to blood pigments. Although this was only one aspect of Hoppe's work, it may be noted that Miescher was quite independent of Hoppe in his conceptions at the time when he began his studies on another type of cells in a pathological liquid of the human body, namely, pus. Going back to the general evolution of biochemistry we should state that the appointment of Hoppe by Virchow was the result of the influence of Justus Liebig (1803-1873), who, within the space of a few years, had established in Giessen, near Frankfort, a magnificent center of instruction and research in chemistry. During the second half of his life Liebig suddenly changed his program from the analysis of artificial substances to the investigation of plants and animal liquids. The first new knowledge of the principle of metabolism in nature, according to Virchow, should be applied to pathology in order to put hematology on a firm foundation. From Liebig's contacts with French chemists—here the mediation of Alexander von Humboldt (1769-1859) plays an important rôle—the spark had passed over to Germany, and the work of Miescher is one of the fruits that the second generation brought to science. From this time on, the ascent of biochemistry was to be much more rapid, aided by scientists like the Nobel Laureate, Albrecht Kossel (1853-1927), who continued successfully what Miescher had begun.

It is typical of Miescher that when he arrived in Tübingen at Eastertime in 1868, he did not go directly to the laboratory of Hoppe, but prepared himself first in the techniques of organic chemistry by working in the chemical institute. According to the well-known physical chemist, Wilhelm Ostwald (1853-1932), Miescher undoubtedly belongs to the so-called classic

type of investigator, namely, one who proceeds systematically along a proposed line straight ahead to a certain goal and who is very cautious about communicating his results. We see in his work features similar to the discoveries of Michael Faraday (1791-1867)—of course on a lesser plane—and to the amazing career of Hermann Helmholtz (1821-1894).

I am sorry not to have the opportunity to give a more detailed outline of the status of chemical biology at that time, but it may be said that Miescher entered quite a new area of research—the investigation of the chemical structure not only of tissues but also of single cells, and even the parts of them. If we glance briefly at the very detailed *Geschichte der physiologischen Chemie* of the Viennese chemist, Fritz Lieben (1935), we can easily see that in the different fields, whether it be compounds of cells or certain laws of metabolism, Miescher opened new patterns of research. This fact explains not only the difficulties of method but also the hard problem of finding the starting points for the theoretical understanding of his observations. These facts, plus his early death, determined Miescher's position as an instigator of new types of research—a pioneer in the finest meaning of the word—rather than a man of great and completed achievements.

In the spring of 1871, near the end of the war between the French and Germans, the scientific world eventually became familiar with the first contributions of the young doctor. He had finished his paper almost two years previously in the laboratory at Tübingen. It was there he had succeeded, after lengthy preparations, in isolating (by artificial digestion through pepsin) the nucleus of the pus cell from the protoplasm and had discovered in its substance the representative of a new group of entities, which he called "nucleine." These compounds contained phosphorus in fixed form and were capable of forming salts with alkalies; thus it was an acid (later called nucleinic acid by Altmann). Since this was the most important part of that treatise, one may easily overlook the fact that also in the cell body Miescher found five albuminous substances and lecithin. Meanwhile, the earlier biochemists had investigated the chemical compositions of the organs as a whole (G. J. Mulder, K. G. Lehmann, J. E. Schlossberger, E. F. von Gorup-Besanez); for the first time the cytological approach had now become significant in the analysis of tissues.

Histology and biochemistry advanced rapidly during the following decades. While he was still in Tübingen, Miescher included in his investigations the cells of the kidneys and of yeast; for the latter Hoppe had developed the technique and brought forth the first results. The vitelline globes were also examined. In all these cells he found the same nucleine which differed from the first only in the quantity of phosphorus. Also, an analysis of the heads of spermatozoa was carried out, and here Miescher found, besides the nucleine, a new basic compound which he called "protamine" and which was also typed as a salt. This salt of nucleinic protamine could be

produced also in crystalline form. A third substance, the so-called "caryogen" in the nucleus of the cell, contained iron—as Miescher could prove.

His investigations were enlarged more and more. He tried to explain, on an exact basis, the phenomenon that the genital glands of the salmon, during a certain period, grow extensively without any supply of food. Miescher recognized in this growth "the most magnificent and most productive experiment of starvation which nature knows," and through his studies he founded the science of intermediate metabolism. He recognized within the dynamics of metabolism that the substances that build up the testicles and the ovaries were derived from certain muscles of the trunk of the fish in which he observed degenerated fibers. Miescher was able to discover the formal causes of this "liquidation," as he called this phenomenon; they consisted of a decreased supply of blood to the peripheral areas which arose from a diminishing of blood pressure. Miescher followed the course of the different properties withdrawn from the muscle and noted several stages of maturation in the spermatozoa during which certain chemical transformations took place.

Miescher made use of this opportunity for a histological examination of the spermatozoa. He also carried out chemical studies on the eggs of the frog, which should clear up the process of impregnation.

Finally, we should mention a field of Miescher's work which won the interest of a few young doctors—among them his successor, Rudolf Metzner —namely, the respiration and the adaptation of the blood cells in higher climates. These investigations should put on a more clinical basis the earlier results reported in the classic book of Paul Bert (1833-1886). Miescher's differentiation between the "apnoea vera" and the "apnoea spuria" contributed much to the clarification of concepts in this complicated matter. The increasing of content of hemoglobin and red blood cells in higher altitudes he called "the finest regulation which is known up to this time in vegetative physiology."

Let me conclude my brief sketch with the sympathetic words of Carl Ludwig, written to Miescher toward the end of Miescher's life: "As grievous as it may be to be so sick, to you remains the consolation of having performed imperishable acts. You have made accessible a substantial analysis of the centre of all organic life, and as often during the next centuries as the cell and your work is scrutinized and exposed, the grateful descendant will remember you as a research pioneer."

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(For complete references see: *Gedenkschrift,* etc., pp. 39-41.)

STUDIES ON THE STABILITY OF LYOPHILIZED BCG VACCINE*†

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The application of lyophilization to the preservation of BCG, in an effort to combat the rapid loss in allergenic potency of the liquid vaccine, has presented important technical difficulties. Although the effectiveness of lyophilized BCG in producing tuberculin conversion appears to be as great as that of the fresh vaccine,[17, 18, 21] clinical experience has shown that its use engenders quantitatively lessened tuberculin reactions[4, 5] that frequently are temporally delayed.[17, 18, 21] More important, however, is the fact that the protective value of lyophilized vaccines against challenge infection with virulent tubercle bacilli is less than that produced by fresh, liquid vaccines.[4, 5]

Inasmuch as the allergenic potency of the vaccine has been shown to be a function of the viability of the suspension[5, 9] the failure of dried vaccines has been attributed to the large decrement in viable cells that occurs during the period of lyophilization itself,[1, 5] and the smaller decrease that occurs in the storage period which follows.

Initial attempts at prolonging the effective life of BCG vaccine were made in Russia in 1937.[14] From the start, workers have experienced difficulty, and the tendency has been to use accessory substances along with the vaccine suspension to enhance its stability. Excipients such as glucose,[4, 14, 21] starch,[14] lactose,[9, 14, 16] gelatin,[20] denatured gelatin,[13] serum,[6, 20] sucrose, gum arabic,[21] peptone solution,[9] sodium ammonium pectate, and glutamic, malic, and succinic acids[3] have been tried. It has been suggested recently that Tween-albumin medium, as used for diffuse, submerged growth of *Mycobacterium tuberculosis,* be used as the vehicle for lyophilization.[15] Rosenthal[16] has reported excellent results using a mixture of lactose, asparagin, buffered salts, and glycerine as a suspending menstruum. Reported survival rates vary from 100 per cent[21, 22, 23] to about 14 per cent,[10] immediately following lyophilization. Birkhaug[4] reports a survival rate of 2.83 to 3.26 per cent three to twelve months after freeze-drying.

Preliminary experiments carried out in this laboratory having supported the reports of low survival rates following lyophilization and, further, having indicated that BCG could not be successfully stabilized in the absence of drying excipients a survey has been made of sixty-two adjuvants, embracing

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the general classes of carbohydrates, organic acids, amino acids and their salts, proteins and digests, gums, and miscellaneous reagents, in order to determine if freeze-drying could be accomplished in a manner that would reduce cellular mortality to a minimum. In addition, because of the suggestion of intrinsic difference in vaccine preparations,[12] nine strains of BCG and one strain of the vole bacillus have been studied to determine if any particular strain could withstand the stresses imposed by lyophilization better than others.

Materials and Methods

*Cultures.**

The nine strains of BCG used in this investigation were:

1. *Strain 31:* old vaccine strain, Phipps Institute, Philadelphia, Pennsylvania. (Dr. J. D. Aronson)
2. *Strain 186:* old Norwegian strain. (Dr. J. Bøe)
3. *Strain 425:* Swedish strain. (Dr. A. Wassen)
4. *Strain 805:* strain from the New York State Laboratory, Albany, New York. (Dr. K. Birkhaug)
5. *Strain 826:* strain from the Trudeau Foundation, Saranac Lake, New York. (Dr. A. J. Vorwald)
6. *Strain 858:* strain from the Tice Laboratories, Chicago, Illinois. (Dr. S. R. Rosenthal)
7. *Strain 862:* strain from the Tice Laboratories, Chicago, Illinois. (Dr. S. R. Rosenthal)
8. *Strain 873:* strain from the Pasteur Institute, Paris, France.
9. *Strain B59:* strain from the New York State Laboratory, Albany, New York. (Dr. K. Birkhaug)

The murine strain of tubercle bacillus was:

1. *Strain 170V:* strain from the Dunn School of Pathology, University of Oxford, Oxford, England. (Dr. A. Q. Wells)

As each culture was received, a transfer was made to a slant of Lowenstein-Jensen's medium. Cultures were sealed with paraffin and incubated at 38° C. These constituted the stock cultures.

For use the stocks were transferred to glycerinated water potato medium. Cultures were incubated at 38° C., at such an angle of inclination that the fluid surface formed a plane with the flattened face of the potato slab.

For the preparation of the vaccine, small portions of the veil of growth on the liquid phase of the culture were transferred to flasks of Sauton's medium. It was found convenient to use Erlenmeyer flasks of 125 ml. capacity, containing approximately 50 ml. of medium. After five to six days' incubation at 38° C. the culture covered the surface of the medium as a thin veil. By the ninth or tenth day the surface growth was markedly thickened and puckered. It was cream to yellow in color, depending upon the strain of BCG that was being grown. Growth was harvested on the tenth day, and the vaccine was prepared for lyophilization.

* All of the cultures used in this investigation were supplied through the courtesy of Dr. Joseph D. Aronson of the Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania.

Preparation of BCG vaccine. The procedure to be described represents, in general, the standard method of preparing BCG vaccine suspensions as carried out in this investigation. Modifications of the established technique, as called for in individual experiments, are indicated in the respective protocols.

Bacilli from ten-day-old Sauton cultures were separated by draining off the culture fluid through a sterile Seitz filter in which the asbestos pad had been replaced by a double thickness of Whatman #4 filter paper, cut to appropriate size. The filter was carefully disassembled and the bacillary mass transferred with a minimum of handling to the center of a small pile of sterile filter papers. A heavy weight was placed upon the pile for approximately ten minutes in order to remove excess moisture.

Portions of the bacillary mat were transferred by means of a spatula to a heavy pyrex grinding flask of 1000 ml. capacity, containing one-half kilogram of marble balls. The flask was rotated at 60 rpm. for five minutes. At this time, gross inspection of the bacillary mass showed it to be rather finely divided. Microscopic examination revealed, however, that the suspension was coarsely clumped. Ten ml. of a 50 per cent solution of glucose were added to the flask and rotation was continued for another five minutes. The suspension appeared homogeneous and darkfield microscopy revealed only occasional clusters of bacilli. Sufficient 50 per cent glucose was added to bring the bacterial concentration to 0.2 mg. dry weight of bacilli per ml. of grinding menstruum, as determined spectrophotometrically (vide infra). After an additional five-minute rotation, darkfield preparations were composed for the most part of freely dispersed bacilli.

At the end of the grinding period, portions of the vaccine suspension were transferred into sterile centrifuge tubes and the exact volume was noted. The tubes were centrifuged in the cold (1° C.) for ten minutes at 3000 rpm. The supernatant fluid was decanted and the packed cells were washed with M/15 phosphate buffer at pH 7.0. Washings were repeated five times, after which the cells were resuspended in phosphate buffer to one-tenth of the original volume. The bacterial concentration was thus brought to 2 mg. dry weight of bacilli per ml. of suspension fluid.

Aliquots of the vaccine were mixed with equal volumes of adjuvant solution, bringing the final bacterial concentration to 1 mg. dry weight of bacilli per ml. of suspension fluid. One ml. amounts of the vaccine were delivered from a glass syringe fitted with a 3-inch 19-gauge needle directly into the bottom of five ml. pyrex ampoules (body 60 mm. long, o.d. 15 mm.; stem 60 mm. long, o.d. 8 mm.). In this manner, loss of vaccine due to adherence of drops of the suspension in the stem of the ampoule was avoided. Routine sterility tests were carried out in fluid thioglycollate medium at the end of the period of grinding and after the final washing of the vaccine suspension.

Standardization of vaccine suspensions. For each point to be established on a dry weight curve indicating opacity as a function of the dry weight, it was necessary to disperse a semi-dry weight of bacteria approximately four times as great.[8] The opacity of the suspension was carefully measured using a Coleman Junior spectrophotometer, at a wave length of 520 millimicrons. Five ml. of vaccine suspension were pipetted into an acid-washed, tared centrifuge tube. The bacilli were packed by centrifugation in the cold at high speed. When sedimentation appeared complete, the supernatant was decanted and an additional five ml. of the vaccine suspension were added to the tube. This process was repeated ten times so that eventually the bacterial pellet at the bottom of the tube represented the concentrate from 50 ml. of the vaccine suspension. The centrifuge tube was placed in a desiccator over "Drierite" (anhydrous $CaSO_4$) and dried to constant weight. All weighings were made to the nearest tenth of a milligram, and the dry weight of the cells, determined by difference, was computed to a ten-ml. volume.

This value was plotted against the percentage light transmission reading. The best straight line was drawn through the experimental points, and the percentage light transmission readings corresponding to the desired milligram weights were determined by inspection. The experimental results are presented graphically in Figure 1.

Technique of lyophilization. Prior to lyophilization, pre-freezing was accomplished by means of external cooling with the aid of a low temperature liquid bath of dry ice and acetone. Ampoules were shell frozen in order to present a maximal surface for evaporation together with a minimal depth of frozen material.

Two lyophile machines were utilized in this investigation. The larger of the two machines conveniently accommodated 62 ampoules, the smaller, 46. An oil-sealed, high vacuum pump of the rotary type (Cenco Megavac) was used in drawing the vacuum, the extent of which was measured with a standard McLeod gauge. Water vapor was removed by condensation at low temperature. The heat necessary for desiccation was supplied by convection from the surrounding air.

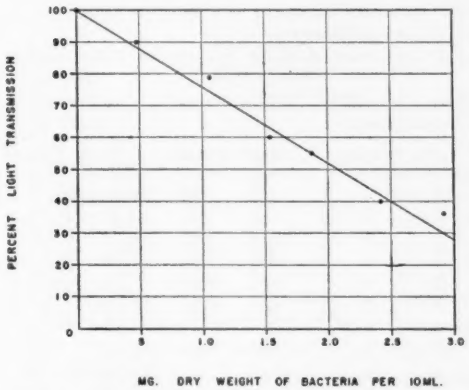

FIG. 1. Percentage of light transmission as a function of dry weight of bacteria.

In practice, a vacuum of 0.05 to 0.1 mm. Hg on "blank suction" was attained before any of the ampoules were attached to the machine. When this level was reached, the shell-frozen ampoules began at once. All dryings were carried out at a level of 0.015 mm. Hg. Leaks in the system were indicated by failure to obtain a sufficiently high vacuum within a reasonable length of time, or by a fall in the vacuum as indicated by the McLeod gauge. Defective ampoules were located with a high frequency spark coil tester of the Tesla type.

The length of time necessary for all condensation on the outer walls of the ampoules to disappear was noted, and then one-half that number of hours additional was allowed to reduce the moisture content within the ampoules to the proper final level of approximately 1 per cent. At the end of the period of desiccation the vacuum pump was turned off and the ampoules were charged with dry nitrogen. They were then sealed with an oxygen flame using a standard Hoke torch fitted with a cross fire attachment.

Method of titration. Independent, duplicate titrations were carried out in Youman's medium to determine the highest dilution to which growth of freshly prepared and lyophilized vaccine suspensions would occur. Serial ten-fold dilutions of freshly prepared or reconstituted lyophilized vaccines were made using separate 1 ml. delivery pipettes and after pipetting the mixture up and down ten times. Dilutions were carried out to a level of 10^{-11}, and after a specified period of time, the greatest dilution at which growth occurred was noted. Only in exceptional instances was there a disparity in the results of duplicate titrations. All culture tubes were provided with aluminum caps in order to obviate any possibility of inhibitory substances becoming incorporated in the medium through the use of cotton plugs.[8]

Experiments

The effect of hydrogen ion concentration upon the preservation of lyophilized BCG. The standard technique was modified in that an eleven-day-old Sauton culture of BCG, strain 858, was ground in M/15 phosphate buffer at pH 7.0 for 25 minutes. The bacterial concentration was brought to 0.2 mg. dry weight of bacilli per ml. of grinding menstruum. Twenty ml. aliquots were pipetted into sterile test tubes which were centrifuged in the

Table 1

The Effect of Hydrogen Ion Concentration upon the Preservation of Lyophilized BCG (The Figures Represent the Negative Log_{10} Titer of Growth)

Series no.	pH	Immediately after lyophilization		After 8 weeks' storage at 4° C.		After 8 weeks' storage at 38° C.	
		3 weeks	5 weeks	3 weeks	5 weeks	3 weeks	5 weeks
1	4.48	2	3	1	2	No growth	1
2	5.08	2	3	2	2	No growth	1
3	5.59	2	3	2	3	1	1
4	6.11	3	4	2	3	1	2
5	6.50	3	5	3	5	2	2
6	7.05	3	7	2	6	3	5
7	7.42	3	6	3	5	1	1
8	8.12	2	3	2	3	1	1
9	8.56	2	3	2	2	No growth	1
Control*	7.05	5	9				

* Unlyophilized.

cold at high speed until sedimentation appeared complete. The supernatant fluid was decanted, and the bacterial pellet was twice resuspended and washed in phosphate buffer solutions at a pH at which the drying was to be carried out. After the final washing, the bacilli were resuspended in one-fifth volume of appropriate buffer solutions. Of the four ml., three were utilized for lyophilization, as previously described, and one for determination of the actual pH of the bacillary buffer mixture, using a Beckman Model G laboratory pH meter. Immediately after lyophilization, one series of ampoules was opened and the dried vaccine reconstituted in 0.9 per cent saline. Titrations to determine the highest dilution at which growth would still occur were carried out in Youman's medium. Of the remaining two series of ampoules, one was stored for a period of eight weeks in the refrigerator at 4° C., and the other in a laboratory incubator at 38° C. for a similar period of time. The highest dilution at which growth occurred was determined at three and five weeks. The results are summarized in Table 1.

Reference to the table will indicate that there was decreased growth on either side of neutrality. Though the decrement was unimportant when the vaccine was used immediately after lyophilization, or even after eight weeks storage at 4° C., the loss in viable elements became disproportionately large when the ampoules were exposed to thermal stress.

The effect of the age of the culture upon the ability of lyophilized BCG to withstand storage after lyophilization. Ten flasks of Sauton's medium were inoculated on the same day with portions of the veil from a glycerinated water potato culture of BCG, strain 873. On the 8th, 10th, 15th, 20th, and 25th day of culture the contents of two flasks were pooled, harvested, and lyophilized according to standard technique. Sodium glutamate was used as the drying adjuvant, since other experiments carried out in this investigation had indicated that it offered a high degree of protection against thermal stress. One series of ampoules was opened immediately after lyophilization and reconstituted in 0.9 per cent saline. Titrations to determine the highest dilution at which growth still occurred were carried out after three weeks' incubation at 37° C. Similar procedures were applied to ampoules stored for eight weeks at 4° C., and for those stored at 38° C.

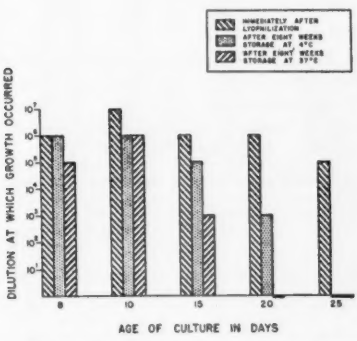

FIG. 2. The effect of the age of the culture upon the ability of lyophilized BCG to withstand storage at refrigerator and incubator temperatures.

The results, which are represented graphically in Figure 2, indicate that with increasing age of the culture there was a decreasing ability to withstand storage after lyophilization. The loss in viability was most marked when the ampoules were stored at 37° C., although after 25 days of culture no viable elements could be recovered from lyophilized material that had been stored at either refrigerator or incubator temperature. The losses do not appear to be due to decreased viability of the culture since there was a variation of only one tube in the highest dilution to which growth occurred from the youngest to the oldest culture, with respect to unlyophilized material. This loss does not appear to be related to a decreased vitality of the strain since the initial loss on lyophilization never exceeded an expected one or two dilution tubes.

The influence of room temperature on the viability of the vaccine suspension. Inasmuch as the preparation of the vaccine suspension required a considerable length of time, experiments were conducted to determine if

there was an important loss of viable elements resulting from prolonged standing at room temperature.

BCG strain 425 was used for this study. Vaccine suspensions were prepared in quadruplicate by the usual technique, but were not lyophilized. Three suspensions were permitted to stand at room temperature. No attempt was made to reduce the ordinary illumination of the room, but the vaccines were shielded from direct sunlight. The first suspension was not altered in any manner. To the second was added 0.1 per cent by volume of a 10 per cent solution of Armour's bovine albumin, Fraction V, and to the third 1 per cent by volume of a 10 per cent sucrose solution. The fourth vaccine was unaltered, but was stored at refrigerator temperature. Titrations were carried out immediately after the preparation of the vaccines, and at four, eight, and twenty-four hours. The results are summarized in Table 2.

TABLE 2

THE EFFECT OF PROLONGED STANDING AT ROOM AND REFRIGERATOR TEMPERATURES UPON THE VIABILITY OF VACCINE SUSPENSIONS (THE FIGURES REPRESENT THE NEGATIVE Log_{10} TITER OF GROWTH)

Nature of vaccine	*Time of titration*			
	Immediate	*4 hours*	*8 hours*	*24 hours*
Unaltered, room temperature	8	8	7	5
Bovine albumin added; room temperature	8	8	8	7
Sucrose added; room temperature	8	8	8	6
Unaltered; refrigerated	8	8	8	8

It may be seen that for a period of four hours there was no loss in any of the four samples. At the end of eight hours the first sample showed a ten-fold decrease in titer and this increased to a thousand-fold loss in viable elements at the end of 24 hours. In the presence of bovine albumin and sucrose the loss at 24 hours was ten-fold and one hundred-fold respectively. Only the refrigerated vaccine was able to maintain its titer.

The degree of dispersion was determined for each preparation at the time titrations were made. Differences of a magnitude which could have been construed to have been significant in the production of the variable titers were not obtained in any instance.

The effect of bacterial dispersion upon the efficiency of lyophilization. To determine if efficiency of drying was a function of the degree of dispersion of the vaccine suspension, equivalent weights of BCG, strain 873, were ground in the presence of phosphate buffer, at varying rates of rotation for

equal periods of time. At the end of the grinding periods, smears were prepared and the percentage of freely dispersed bacilli was noted. Lyophilization was carried out in the presence of sodium glutamate, and, in the usual manner, decimal dilutions were made in Youman's medium to determine the highest dilution at which the vaccine suspension would still grow. Ampoules were stored in the refrigerator and at incubator temperature for a period of eight weeks. The results are presented graphically in Figure 3. It may be noted that the poorer the dispersion, the poorer the ability of of the vaccine suspension to withstand lyophilization. A disproportionate decrease in the thermal stability of poorly dispersed, glutamate dried suspensions is evident.

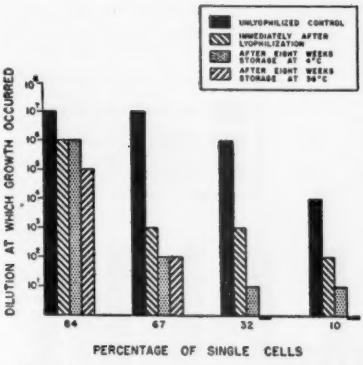

FIG. 3. The effect of bacterial dispersion upon the efficiency of lyophilization.

The importance of the drying adjuvant in combatting the loss of viable elements during lyophilization. In this experiment, all available strains of BCG, and a strain of the vole bacillus were studied with respect to their

TABLE 3

NEGATIVE Log_{10} IN TITER IMMEDIATELY AFTER LYOPHILIZATION, IN THE PRESENCE OF A SELECTED SERIES OF ADJUVANTS

Adjuvant	*Number of strain*									
	858	*425*	*805*	*31*	*873*	*B59*	*826*	*862*	*186*	*170V*
Galactose d	2	2	1	3	2	3	2	4	1	3
Glucose	1	1	1	2	2	1	1	2	1	2
Sucrose	2	1	3	2	2	3	3	2	3	2
Lactose	1	1	1	2	1	2	2	2	1	2
Sodium glutamate	1	2	3	2	1	1	1	2	1	2
Sodium aspartate	2	1	2	3	2	2	4	1	2	3
Gelatin	5	5	4	6	5	7	4	3	5	6

ability to withstand the stress of lyophilization when dried in the presence of various adjuvants. The agents used for drying embraced the general classes of carbohydrates, organic acids, amino acids and their salts, proteins and digests, and gums. A group of adjuvants not easily classified were included under the general heading, miscellaneous. Unless otherwise noted, all strongly acidic or basic compounds were neutralized prior to use.

Each of the adjuvants was used for the preparation of a vaccine suspension of the ten separate cultures, and the negative $\log_{10}$ decrement in titer, i.e., the difference in the titer of growth between the unlyophilized and lyophilized vaccine suspensions, was determined for each of the ten strains. Table 3 gives the negative $\log_{10}$ decrement in titer for the six adjuvants found to be most useful, and for one adjuvant considered to be poor, with reference to each particular strain. It is evident that there was no marked

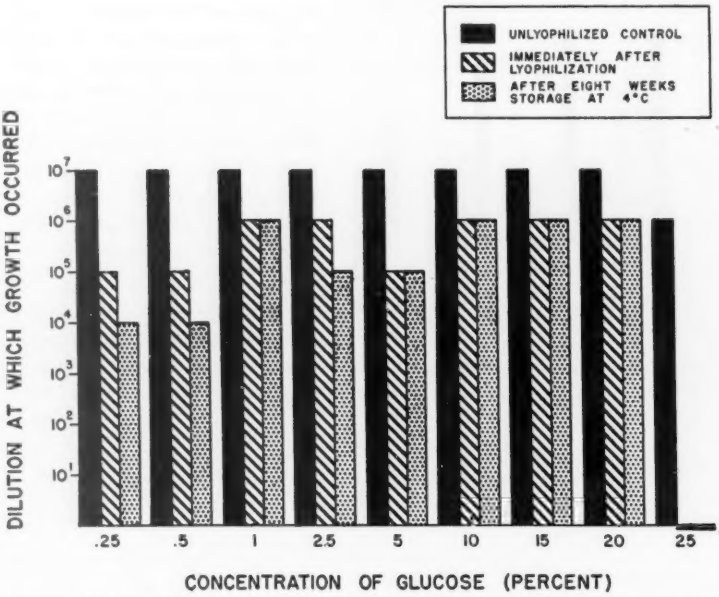

FIG. 4. The effect of varying concentrations of glucose upon the ability of vaccine suspensions to withstand lyophilization.

strain difference in the ability to withstand lyophilization, since the variation in the degree of recovery, with any particular adjuvant, was not marked. An adjuvant which was good afforded protection to all strains, and conversely, a poor adjuvant was poor with respect to all strains.

An index of efficiency was determined for each adjuvant by adding together the $\log_{10}$ decrements in titer for the ten strains of mycobacteria. For the purpose of this investigation, any adjuvant whose index of efficiency was 25 or lower was considered worthy of further study.

The adjuvants used, their final concentrations within the ampoules, and their indexes of efficiency are listed in Table 4. It may be noted that of the six adjuvants that appeared to offer a significant degree of protection to the

TABLE 4

THE ADJUVANTS, THEIR CONCENTRATIONS AND INDEXES OF EFFICIENCY

Adjuvant	*Final concentration in ampoule (%)*	*Index of efficiency*
Carbohydrates		
Mannitol d	5	38
Fructose	5	28
Mannose d	5	31
Galactose d	5	*23*
Xylose l	5	35
Inulin	5	33
Dextrin	5	30
Raffinose	5	41
Dulcitol	1	44
Salicin	1	29
Inositol	1	37
Sorbitol d	1	47
Adonitol	1	48
Starch, soluble	0.5	50
Xylose d	1	38
Maltose	5	34
Glucose	5	*14*
Sucrose	5	*22*
Lactose	5	15
Organic acids		
Succinic acid	0.5	45
p-amino benzoic acid	0.15	40
Adipic acid	0.5	47
Malonic acid	0.5	43
Glutaric acid	0.5	34
Ascorbic acid	0.5	50
Sodium citrate	5	42
Azelaic acid	0.1	50
Valeric acid	0.5	43
Pimelic acid	0.5	48
Gluconic acid	0.5	37
Nicotinic acid	0.5	42
Amino acids, and their salts		
Tyrosine	0.5	49
Cystine	0.5	47
Histidine	0.5	47
Sodium glutamate	1	*16*
Alanine	0.5	33
Glutamic acid*	0.5	50
Arginine	0.5	50

* Unneutralized.

Adjuvant	*Final concentration in ampoule (%)*	*Index of efficiency*
Asparagin	0.5	31
Lysine	0.5	43
Aspartic acid*	0.5	47
Sodium aspartate	1	22
Glycine	0.5	42
Proteins and digests		
Trypticase	1	56
Amigen	5	52
Neopeptone	0.5	44
Mucin	0.5	53
Gelatin	0.5	50
Gums		
Arabic	0.25	30
Tragacanth	0.25	34
Ghatti	0.25	36
Miscellaneous		
Brain heart infusion	1.85	46
Creatinine	0.5	51
Liver extract	1	58
Guanidine sulphate	0.5	51
Water, distilled	...	50
Saline	0.85	42
Sulfathiazole	0.5	52
Kaolin	1	41
Horse serum	5	30
Charcoal	5	53
Urea	0.5	50

* Unneutralized.

bacilli, four were carbohydrates (galactose, glucose, sucrose, lactose), and two were salts of organic acids (sodium glutamate, sodium aspartate).

The optimal concentration of adjuvant solutions to be used in lyophilization. This experiment was carried out using BCG strain 862. Since it had been noticed that high concentrations of carbohydrates were exceedingly difficult to dry with any degree of success due to "bubbling" and loss of vaccine through the stem of the ampoule, and in view of the report that high salt concentrations were detrimental to survival,[7] experiments were undertaken to determine the optimal amounts of selected adjuvants to be used in lyophilization. As a typical carbohydrate, glucose was chosen; as a typical salt, sodium glutamate was selected. Vaccine suspensions were prepared and

lyophilized in 0.5, 1, 2, 5, 10, 20, 30, 40, and 50 per cent glucose. The final concentration within the ampoules was one-half of these values. Similarly, suspensions were prepared and lyophilized in the presence of 0.1, 0.2, 0.25, 0.5, 1, 2, 5, and 10 per cent sodium glutamate. Again, the final concentration within the ampoules was one-half of these values. One series of ampoules, consisting of both glutamate and glucose dried vaccines was opened immediately after lyophilization, another glucose dried series after eight weeks' storage at 4° C., and a third glutamate dried series after a similar period of

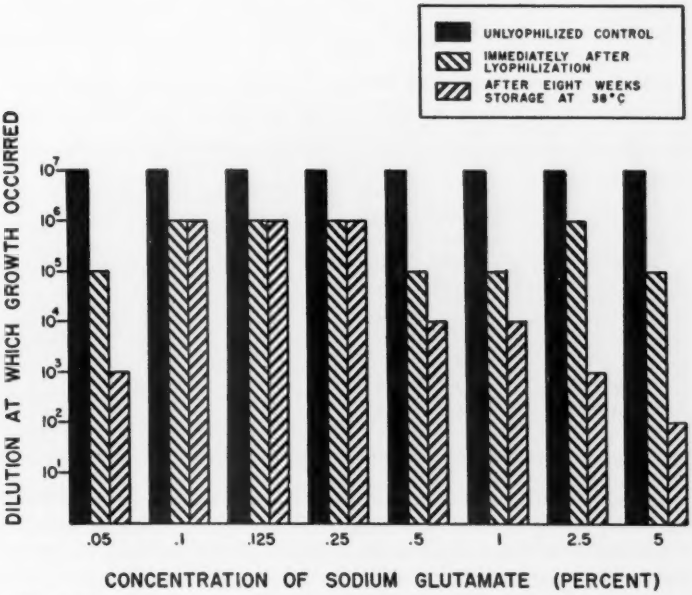

FIG. 5. The effect of varying concentrations of sodium glutamate upon the ability of vaccine suspensions to withstand lyophilization.

storage at 38° C. Titrations were carried out in Youman's medium as previously described. The results are presented graphically in Figures 4 and 5.

It may be seen that with carbohydrate dried material there was little difference in the efficiency of drying, regardless of the concentration of the carbohydrate used, except for the extremely low final concentrations of 0.25 and 0.5 per cent. However, in the case of glutamate dried material, there was a definite optimal range of salt concentrations, and in agreement with previously published data, high salt concentrations were found to be detrimental to survival.

The ability of lyophilized BCG to withstand prolonged storage at refrigerator and incubator temperatures. This investigation was carried out with BCG strain 858. Vaccine suspensions were lyophilized in the presence of all adjuvants according to standard technique. Titrations were carried out upon the unlyophilized vaccine and upon the lyophilized product immediately after freeze-drying. Corresponding ampoules were stored in the refrigerator at 4° C., and in a laboratory incubator at 38° C. The greatest dilution at which growth still occurred was again determined after three and ten months. The results for seven of the adjuvants tested are given in Table 5.

TABLE 5

THE ABILITY OF LYOPHILIZED BCG TO WITHSTAND PROLONGED STORAGE AT REFRIGERATOR AND INCUBATOR TEMPERATURES (THE FIGURES REPRESENT THE NEGATIVE Log_{10} TITER OF GROWTH)

Adjuvant	*Unlyophilized control*	*Immediately after lyophilization*	*Length of storage*			
			3 months		*10 months*	
			4° C.	*38° C.*	*4° C.*	*38° C.*
Galactose d	6	4	3	3	3	1
Glucose	7	6	6	1	5	No growth
Sucrose	7	5	5	2	4	No growth
Lactose	7	5	4	2	4	1
Sodium glutamate	6	5	4	4	4	4
Sodium aspartate	8	5	5	4	5	5
Gelatin	6	1	1	No growth	No growth	Not done

It may be noted that the carbohydrates offered the greatest protection to the bacilli during a period of prolonged storage at refrigerator temperature. A few amino acids and organic acids seemed to afford protection at refrigerator temperatures, but the proteins, gums, and miscellaneous reagents were poor in this respect.

Adjuvants which afforded greater protection to the bacilli, under conditions of thermal stress, than did water alone, were considered worthy of further study. Of all adjuvants tested, only three (sodium glutamate, sodium aspartate, ascorbic acid*) were capable of maintaining the viability of vaccine suspensions after prolonged storage at incubator temperatures.

The ability of a combination of adjuvants to protect bacilli during prolonged periods of storage and against thermal stress. An examination of the experimental results indicated that the most favorable adjuvant for pro-

* Titrations carried out after drying in the presence of ascorbic acid show a marked inhibition of growth at low dilutions. However, when drying is carried out at a dilution of ascorbic acid that is not inhibitory, the protective effect is no longer obtained.

longed storage of vaccine suspensions in the cold was glucose, and that the adjuvant offering the greatest protection against thermal stress was sodium glutamate. It was decided, therefore, to determine whether or not a combination of the two adjuvants could be used to prepare a vaccine suspension that would be stable under both of these conditions. BCG strain 862 was lyophilized in a mixture of optimal concentrations of glucose and sodium glutamate. Titrations were carried out immediately after lyophilization and after storage periods of three and ten months. The results are given in Table 6.

It may be seen that the preservation of the vaccine in the cold, by the use of combined adjuvants, was poorer than when glucose or sodium glutamate

TABLE 6

THE ABILITY OF A COMBINATION OF ADJUVANTS TO PROTECT BACILLI DURING PROLONGED PERIODS OF STORAGE AND AGAINST THERMAL STRESS (THE FIGURES REPRESENT THE NEGATIVE Log_{10} TITER OF GROWTH)

Nature of adjuvant	*Unlyophilized control*	*Immediately after lyophilization*	*Length of storage*			
			3 months		*10 months*	
			4° C.	*38° C.*	*4° C.*	*38° C.*
Glucose and sodium glutamate	7	6	3	2	2	1
Glucose	6	5	5	1	5	1
Sodium glutamate	6	5	5	4	5	4

was used alone, and that preservation under conditions of thermal stress was poorer than when sodium glutamate was used alone.

The ability of BCG repeatedly subcultured upon Sauton's medium to withstand lyophilization. It has been shown previously[15, 28] that serial subculture of BCG on Sauton's medium causes a loss in vitality of the culture, although introduction of a single passage on potato is sufficient to restore the vitality of the strain. Loss of vitality is manifested by delayed initial growth, production of a hydrophilic bacillary mat, decrease in the size of the bacilli, and diminution in the allergizing powers of the culture. It was of interest to determine if the decreased vitality of the strain would lead to a decrease in the ability to withstand the stresses of lyophilization.

BCG strain B59 was chosen for the experiment. An initial transfer from a glycerinated water potato culture was made to Sauton's medium. Every tenth day thereafter, for a period of 100 days, a serial subplant was made from the Sauton culture to a new flask of Sauton's medium. Titrations were carried out upon each culture to determine the highest dilution to which growth would occur, and, at the same time, the cultures were lyophilized in the presence of 20 per cent glucose (final concentration 10 per cent).

Ampoules were opened for titration immediately after lyophilization. A comparison was made of the highest dilutions at which the unlyophilized and lyophilized bacilli grew.

After the tenth serial passage upon Sauton's medium, the culture was regrown on glycerinated water potato medium. A portion of the veil from the new culture was transferred to Sauton's medium, and the new first

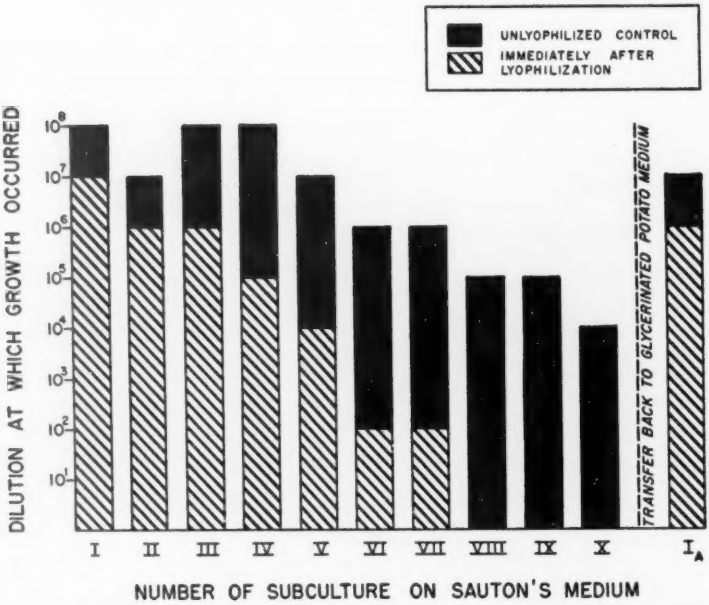

FIG. 6. The effect of repeated subculture in Sauton's medium upon the ability of BCG to withstand lyophilization.

generation was designated Ia. A comparison was made of the highest dilutions at which the unlyophilized and lyophilized Ia cultures grew.

The results of the experiment are presented in Figure 5. It is evident that with repeated subculture there was a marked decrease in the viability of the culture. Coincident with this loss there was a disproportionate decrease in the ability of the culture to withstand lyophilization. Although the initial loss after lyophilization in the presence of glucose may be expected to encompass one, or at the most two, dilution tubes, it may be noted that after the fourth generation losses exceeded expectation, and that after the eighth passage, there was no recovery of viable elements from the lyophilized material.

It is of further interest to note that a single passage back to potato was sufficient to restore the ability of the strain to withstand lyophilization, since the loss suffered by culture Ia following lyophilization amounted to only a single dilution tube.

Discussion

The ultimate object of this investigation was to secure a stable allergenic preparation of BCG. Although progress was made in the direction of stability, it has not been possible, up to this time, to assay the allergenic properties of the vaccine suspensions. In a discussion of the results it seems pertinent to indicate the difficulties inherent in this type of work, and to point out the particular shortcomings that appear here.

Because the allergenicity of BCG vaccine has been shown to be a function of the degree of viability of the bacterial suspension, attention has been devoted primarily toward the development of a method for obtaining lyophilized cultures with the highest possible number of viable cells. It must be admitted, however, that the assumption cannot be made that the viability of the vaccine is the only factor involved in the degree of allergenic potency. Lyophilized vaccine suspensions produced in Japan show relatively high levels of viability, but less than 50 per cent of such preparations cause tuberculin conversion. In addition, there appears to be a qualitative difference in the nature of a positive response to tuberculin, in that the reaction produced shows a marked increase in inflammation, but a decreased amount of induration.[3]

The method of assay used in this investigation was a determination of the highest dilution at which vaccine preparations gave growth in Youman's medium, both before and after lyophilization. The same method was utilized in all assays so that the results are comparable, and are an index of the effect of different treatments on the relative numbers of viable cells. Although the method gave no indication of the absolute number of viable cells in the vaccine suspensions it was deemed satisfactory for a comparative evaluation of the effect of different procedures on viability.

The principal source of error in the assay would arise from those procedures which tended to aggregate cells at any time during vaccine preparation, lyophilization, or storage, or which tended to prevent dispersion of the vaccine on later dilution. Such aggregation would be reflected as a diminished population in subsequent titrations of growth and, in the absence of corrections to account for the clumping of the vaccine, could only be interpreted as an indication of a low index of efficiency for the adjuvant concerned.

It must also be realized that any lyophilized vaccine contains cells in three states of viability: living cells, dead cells, and an intermediate group that may live or die, depending upon the conditions under which the suspension is reconstituted. These represent potentially viable cells. Throughout these

experiments, vaccine suspensions were reconstituted in 0.9 per cent saline. It is possible that the use of saline did not permit full realization of the potential population, and that by the use of other diluents growth would have been secured at higher dilutions. Quite obviously, the determination of the highest titer of growth is a function of the adequacy of the technical methods employed for the demonstration of viability.

Errors in evaluating particular procedures or adjuvants might also have arisen because of lack of knowledge of special conditions affecting performance. Thus, because of the large number of adjuvants tested, it was not feasible to determine for each compound the optimal concentration to be used in lyophilization. Examination of the results summarized in Figure 5 reveals a narrow range of protective concentrations of sodium glutamate. It is possible that potentially valuable adjuvants have been neglected in this study because screening tests carried out at a single concentration showed them to disadvantage.

It has also been difficult to assess the importance of aggregation or dispersion of the bacterial suspension. Differences in the ability to withstand the stresses imposed by lyophilization have been correlated with rather gross changes in the percentage of freely dispersed bacilli. If it is granted that the aliquot removed from the vaccine suspension for the determination of the percentage of freely dispersed bacilli was, in fact, representative of the vaccine suspension, then it would appear that it is easier to stabilize the single bacterial cell than the clump of bacterial cells. This experimental finding may have its basis in the difficulty with which an adjuvant reaches the interior of a bacterial clump. Equally plausible is the contention that dispersion is effective because it permits more rapid dehydration of the vaccine suspension and thus prevents the formation of ice crystals within the cell. Nevertheless, the possibility remains that a clumped bacterial suspension might act as a depot after inoculation and thus give rise to a heightened and sustained allergenic stimulus.

Finally, it has not been possible to relate definitely the chemical structure of the drying adjuvant to its protective capacities. Of seven adjuvants that afforded protection to the bacilli, three were hexoses, one a hexose derivative, one a disaccharide, and two, salts of organic acids. Examination of the experimental data will indicate, however, that, in many cases, chemical analogues varied rather widely in the degree of protection afforded to the bacilli. Thus, the indexes of efficiency for the sodium salts of glutamic acid and glutaric acid were 16 and 34 respectively.

Despite these many objections, however, it seems likely that the following points have been established.

There was no significant variation in the ability of the different strains of BCG, or of the vole bacillus, to withstand lyophilization. The success of freeze-drying was dependent upon the suspending vehicle, rather than upon inherent differences in the strains.

Loss in viability of the vaccine suspensions occurs at three times: first, during the period of vaccine preparation, prior to lyophilization. This initial loss could be eliminated or minimized by refrigeration of the vaccine or by the addition of serum albumin. It might be well to note at this point that refrigeration is, of course, preferable to the addition of serum albumin, because of the potential danger of inducing serum sickness. Further loss during the period of lyophilization could be combatted by drying in the presence of each of a small number of adjuvant solutions. Of 62 adjuvants investigated only six offered protection to the bacilli during freeze-drying. Four of these were carbohydrates (galactose, glucose, sucrose, lactose) and two were salts of organic acids (sodium glutamate, sodium aspartate). The carbohydrates protected the bacilli during prolonged periods of storage in the cold, but were inefficient under conditions of thermal stress. Salts of organic acids were less efficient than carbohydrates, but did confer some protection against thermal stress.

Although glucose was the most suitable adjuvant for combatting the loss of viable elements during the period of lyophilization, the compound presented technical difficulty due to its tendency to "bubble" during the freeze-drying process. From the standpoint of practicality, lactose, which is relatively less efficient than glucose, might be preferred inasmuch as it does not "bubble" during lyophilization and has a faster rate of solubility on reconstitution of the vaccine suspension.

The degree of protection afforded by glucose was independent of concentration over a wide range; sodium glutamate was protective only over a limited range of concentrations.

Although carbohydrates were desirable as adjuvants for prolonged preservation of vaccines, these compounds offered little or no protection when the ampoules were exposed to thermal stress. Only three compounds were demonstrated to protect bacilli stored at incubator temperature. These were sodium glutamate, sodium aspartate, and ascorbic acid. The last, however, because of its aberrant behavior, as indicated earlier in this paper, cannot be recommended for use. The other two compounds, sodium glutamate and sodium aspartate, offered marked protection against thermal stress, but were not as efficient as carbohydrates when storage could be effected at refrigerator temperature.

When carbohydrates and salts of organic acids were mixed, the resultant adjuvant solution did not suffice to stabilize the vaccine against both prolonged periods of storage in the cold, and storage under conditions of thermal stress. Indeed, the inherent value of each of the individual components was lessened, with consequent degradation of the integrity of the lyophilized product.

Lyophilization was best accomplished at a neutral pH. Loss in viability of vaccine suspensions dried at hydrogen ion concentrations on either side of neutrality became disproportionately large when freeze-dried prepara-

tions were stored at incubator temperature. It is felt that the importance of hydrogen ion concentration has been too little appreciated in the past, a fact attributable both to the length of time for its effect to be noticed in culture, and also because the potentially adverse conditions of thermal stress have been assiduously avoided in storage whenever possible.

The efficiency of lyophilization was related to the initial dispersion of the vaccine suspension. There was a disproportionate decrease in the ability of poorly dispersed vaccines, in adjuvant solutions, to withstand lyophilization. The use of "Tween 80" in the suspending menstruum to encourage dispersed growth should make it possible to eliminate the lethal effects of prolonged grinding. It is to be hoped that the preliminary reports on the use of "Tween 80" in the suspending vehicle[15] will soon be followed by a detailed analysis.

"Lyophile" preparations of old cultures (20 to 25 days) showed a decreased ability to withstand storage at both refrigerator and incubator temperatures. The loss in viability was most marked when the ampoules were stored at 37° C. However, with 25-day-old cultures, no viable cells were recovered from lyophilized material kept at either refrigerator or incubator temperatures. It would seem quite evident that if a high degree of viability is to be expected in a lyophilized preparation, the culture from which the vaccine is prepared must itself contain a high percentage of viable cells. Traditional Calmette technique calls for the use of a 21- to 30-day-old BCG culture, but such a culture has long passed its period of exponential growth and is composed predominantly of dead cells. The use of such a culture is open to question.

Repeated subculture of BCG on Sauton's medium led to a marked decrease in the vitality of the culture, and a disproportionate decrease in the ability of the culture to withstand lyophilization. Vitality could be restored by a single passage back to glycerinated water potato medium. It would appear wise to limit the number of consecutive transfers of the BCG culture on Sauton's medium, and any delay in the appearance of initial growth should be taken as indication of the necessity of repassaging the culture back to a potato medium.

It has not been possible in this study to confirm the reports of relatively slight loss of viable elements during lyophilization.[21, 22, 24] Claims of high recovery rates after lyophilization must be regarded with some reserve and must be evaluated in accordance with the techniques employed for the enumeration of the viable elements in a vaccine suspension. The greatest efficiency of drying obtained in this investigation has been 10 per cent.

Because the experimental results indicate clearly the difficulty of stabilizing BCG in the absence of adjuvants, it is of interest to consider what rôle the adjuvant actually may play in stabilization. The theories generally advanced fall into two categories, the physical and the physiologi-

cal. The following may be cited as examples of physical explanations of adjuvant action:

1. The adjuvant solution prevents microtrauma to the bacterial cell by inhibiting the formation, at low temperatures, of ice crystals within the cell.

2. The adjuvant adheres to the cell wall, and, in this manner, renders the cell impermeable.

3. The adjuvant solutions shrink the cell so that it does not undergo plasmoptysis on reconstitution of the dried suspension.

In contrast, the following physiological explanations have been offered for consideration.

1. The adjuvant solution penetrates the cell and by some unknown process, possibly competitive inhibition, manages to suppress the metabolic activities of the cell.

2. The adjuvant exerts a protective effect or "sparing action" upon an essential cell metabolite.

It does not necessarily follow that the explanation of adjuvant action must be either entirely physical or physiological, and, undoubtedly, both factors are operative. However, in the domain of physical explanations, it is difficult to see how the soluble substances which have been demonstrated in this study to exert a protective action, could constitute a somatic barrier to render a bacterial cell impermeable.

Because of the possibility that adjuvants exert their protective action at a metabolic rather than at a structural level it would have been extremely interesting had all of the substances shown to be effective in stabilizing the vaccine suspensions been physiologically active. This was, however, not the case, and one of the best adjuvants, lactose, apparently plays no part in the metabolism of mycobacteria. Similarly, sucrose is not utilized by the organism, and sodium glutamate, the adjuvant that confers the greatest protection against thermal stress, under experimental conditions causes such small increments in the Q_{O_2} of washed cell suspensions that its importance to the metabolic economy of the organism is questionable.

This investigation has, therefore, failed to clarify the nature of adjuvant action.

Summary

An investigation has been undertaken to determine in what manner cellular mortality of BCG might be kept to a minimum during lyophilization. No significant variation in the ability of nine strains of BCG and one strain of the vole bacillus to withstand lyophilization has been demonstrated. The success of freeze-drying has been shown to be dependent upon the age of the culture used for lyophilization, its degree of dispersion, and the initial neutrality of the suspending vehicle. The effect of prolonged standing, at

room and refrigerator temperatures, upon the viability of vaccine suspensions, and of repeated subculture of the BCG strain have been assessed.

Sixty-two adjuvants, embracing the general classes of carbohydrates, organic acids, amino acids and their salts, proteins and digests, gums, and miscellaneous reagents have been investigated with respect to their ability to protect vaccine suspensions during lyophilization and storage and against thermal stress. Of six adjuvants demonstrated to protect bacilli during freeze-drying, four were carbohydrates (galactose, glucose, sucrose, lactose) and two were salts of organic acids (sodium glutamate, sodium aspartate). The protective capacities of the carbohydrates were superior to those of the salts of organic acids when storage could be effected at refrigerator temperature, but were inferior when storage was effected at higher temperatures.

The optimal concentrations of selected adjuvants to be used for lyophilization have been determined. The inability of a combination of adjuvants (glucose and sodium glutamate) to protect bacilli during prolonged periods of storage at refrigerator and incubator temperatures has been demonstrated.

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EVENTS

C.-E. A. WINSLOW LECTURE

November 14, 1952

STATESMANSHIP IN PUBLIC HEALTH. By Leonard Scheele, Surgeon General, U. S. Public Health Service.

[The pupils of Professor Winslow inaugurated a lectureship fund to honor him on his seventieth birthday on February 4, 1947. This is the first of a series.]

Today public health depends more on statesmanship than on techniques. Statesmen, not technicians, cultivate the broad outlook in many spheres. Though health is intimately related to the economy of all individuals, it is difficult to persuade others of the advantage of this mutual security.

One does not have to convince society that chronic disease and the diseases of old age are the problem of all; governing bodies must be convinced. We must be self-critical of our activities, raising our sights to neglected fields. A search must be made for simplified techniques which the general practitioner can use.

Little money was available before the war for medical research. With an increase in public interest came an expansion in investigative projects; government agencies have allocated funds in increasing amounts to universities and hospitals. These co-operative research projects with university schools of medicine are some of our most important partnerships.

J. K. R.

CARDIOVASCULAR STUDY UNIT

December 17, 1952

ANTIBIOTIC PROPHYLAXIS OF RHEUMATIC FEVER. By G. H. Stollerman, Medical Director, Irvington House, Irvington-on-Hudson, New York, and Instructor, New York University College of Medicine, New York.

The effectiveness of chemoprophylaxis of rheumatic fever with sulfonamides and penicillin more than compensates for the accompanying risks of toxicity and production of resistant bacteria. Penicillin will probably prove to be a more successful prophylactic agent than the sulfonamides. The unreliability of oral penicillin administration prompted the utilization of N,N′ dibenzyl-ethylenediamine dipenicillin. Three weeks after a single intramuscular injection of 1,250,000 units of this slowly absorbed compound, average blood levels of 0.07 units milliliter were detectable in 100% of the patients.

J. P. G.

MICROBIOLOGY SEMINAR

January 9, 1953

PROBLEMS RELATED TO COCCIDIOIDOMYCOSIS. By R. A. Vogel, Department of Microbiology, Yale University.

Although the spherule of Coccidioides is invariably found in the tissues of the infected host, current methods of *in vitro* cultivation of the fungus have not provided an adequate technique for culturing large quantities of this

phase of the fungus. Moore (1941) observed limited conversion of the mycelial to spherule form when he inoculated the chorion-allantois of the embryonated hen's egg. Breuck (1951) briefly mentioned the conversion of the arthrospores of this fungus to the spherule stage in the yolk sac of the egg. Further investigation of this problem by the present investigator has shown that using the yolk sac via the window technique of inoculation, large quantities of spherules can be obtained from yolk after extraction of the egg protein with 10 per cent NaCl and centrifugation. A comparison of techniques showed that the blind method of inoculating the yolk was unsatisfactory, leading to the early death of 100 per cent of the eggs and incomplete development of the spherules. It is suggested that inoculation by this method results in an infection involving the shell membrane and chorion-allantois which proves fatal due to hematogenous spread.

The spherule material was found to stimulate antibody production in rabbits, both to egg protein and spherule; however, after absorption of egg protein antibodies, sera from these rabbits still contained antibodies to the spherule. Experiments were carried out to determine the immunizing capacity of this material in guinea pigs. Coccidioidin sensitivity was induced in immunized animals. Immunized and control animals were challenged by aerosal infection with the mycelial phase. Gross and microscopic examination of lung tissue showed the immunized group far more resistant to the infection.

M.-L. J.

YALE MEDICAL SOCIETY

January 14, 1953

OVARIAN AGENESIS IN CHILDHOOD. By H. K. Silver, M.D., Department of Pediatrics, Yale University School of Medicine.

In 1938, Turner described a syndrome in adult women characterized by shortness of stature, sexual infantilism, congenital webbed neck, and cubitus valgus. This condition has been shown to be due to ovarian agenesis. Other manifestations of the disease which may occur include coarctation of the aorta, wide spacing of the nipples, and increased urinary gonadotropins. We have studied several children with this syndrome and have found that the urinary gonadotropins may be markedly elevated even prior to puberty. Other findings noted in some of the children included edema of the feet at birth, osteoporosis, and chondrodystrophy.

Since the physical findings vary among different individuals, a diagnosis may be made with certainty only on the basis of laboratory determination of increased gonadotropins or when examination discloses ovaries with fibrous streaks and an undeveloped uterus.

H. K. SILVER

DEVELOPMENTAL FORMS OF VACCINIA VIRUS. By W. H. Gaylord and J. L. Melnick, Department of Preventive Medicine, Yale University School of Medicine.

The application of techniques for ultra-thin sectioning to the study of the intracellular development of two of the pox viruses has revealed part of a

complex life cycle. Chorio-allantoic membranes of 12-14-day eggs infected with vaccinia and ectromelia, and vaccinia-infected rabbit cornea were studied with the electron microscope.

Developmental bodies, larger and less dense than mature virus, were seen in different stages of maturation. The developmental bodies had a thin limiting membrane and appeared at various times to be "empty," filled with a homogeneous material of low electron density, or half filled with the same material but displaying a very dense central granule. The granule apparently increased in size at the expense of the less dense material.

The developmental bodies were imbedded in a dense cytoplasmic matrix in the case of vaccinia in the egg. Ectromelia in the egg and vaccinia in the cornea produced large, dense structures identified as true inclusion bodies. In such cases the developmental bodies were sometimes seen within disintgrating inclusion bodies. Inclusion bodies or cytoplasmic matrices disappeared with an increase of the mature virus.

W. H. GAYLORD

NU SIGMA NU ANNUAL LECTURE

January 20, 1953

BALLISTOCARDIOGRAPHY. By William Dock, Professor of Internal Medicine, State University of New York, College of Medicine, New York.

Ballistocardiographic experimentation began approximately twenty years ago with the work of Henderson and of Starr. At first only of research interest, recent work shows the ballistocardiograph (BCG) to be of considerable practical value (used of course in conjunction with the usual clinical methods) in assessing the vascular status of "normal" people, in the detection and subsequent assessment of therapy in coronary disease, and in the detection of high output cardiac failure.

There are two essential methods of taking a BCG, although there are many further variations upon the basic themes. The patient is placed upon a table and in one method the table is allowed horizontal motion and it is this motion which is recorded. In the alternative method, the table is held secure and the motion of the patient is directly recorded. The speaker's work has been with the second method, because it is less expensive and because it is applicable to individuals of any size, whereas the moving table method is not applicable to children below 20 kg. in weight. An apparatus making use of a moveable electromagnetic coil and photoelectric cell is placed upon the patient's shins for the actual recording of motion.

Originally, the form of the BCG was thought to be determined by stroke volume, but experiments involving injection with a piston into the arterial system of a cadaver with simultaneous BCG recordings show that not the stroke volume but rather the velocity with which the ventricles begin to eject blood is the important factor in determining the shape of the BCG. It was also once thought that the H wave was determined by auricular contraction, but the fact that the H wave does not change during auricular fibrillation shows that this is not the case.

One precaution must always be observed before making a record—tobacco must be avoided before the record is made, as nicotine will cause

cardiovascular changes which will greatly obscure the record. Amplitudes are normally greater in inspiration than expiration, and considerably greater after exercise in a normal BCG. With diffuse myocardial damage, however, a post-exercise BCG will show little change or even look worse (and so indicate pathology), while the ECG may show no change. With subepicardial damage, though, the post-exercise BCG may look normal while the ECG shows further abnormalities.

Many examples may be given of the practical uses of the BCG. The record of course stems primarily from the force of the heartbeat; from collateral evidence, it is known that the I-J displacement is determined by the force of right ventricular ejection, and the K wave is a measure of velocity of blood flow down the abdominal aorta. Changes in the BCG may be seen in normal pregnancy where the I-J waves vary markedly with respiration because of the increase in venous pooling, venous inflow, and consequently in right ventricular ejection. In coarctation of the aorta, very short K waves are seen. In mitral stenosis, the waves occurring during diastole may be found to be far larger than those during systole. In heart block, the ventricular waves will be increased, as will the left-to-right excursions in a lateral BCG corresponding to the heightened auricular beats. In high-output failure, the BCG shows increased amplitudes. After the cure of beriberi heart disease, the amplitudes decrease because of the decrease in peripheral vasodilation present during disease. The BCG waves will be decreased in congestive failure, but with therapy will show increased excursions before there are any changes in arterial blood pressure; the increases in amplitude corresponding to greater ease of ejection from the right ventricle. A more delicate assessment of therapy is thus available. In angina and myocardial infarct, the BCG may be markedly changed even with minimal ECG changes.

R. G.

MICROBIOLOGY SEMINAR

January 23, 1953

THE BIOSYNTHESIS OF ORNITHINE AND PROLINE. By H. J. Vogel, Department of Microbiology, Yale University.

In a study of biocemical mutants of E. Coli, it was observed that one mutant accumulated a metabolic product which supported the growth of a second. Although the compound was unstable at neutrality, it was found to be stable in acid solution and was tentatively identified as α-glutamic semialdehyde which combines with *o*-aminobenzaldehyde to give a yellow compound. The semialdehyde was synthesized, and the synthetic compound was found to have the same biologic activity as the natural product.

According to Bonner's work with Neurospora, proline and ornithine can be synthesized from glutamic acid, which goes to glutamic-α-semialdehyde and then to pyrrolidone carboxylic acid and proline or to ornithine. However, work with biochemically deficient mutants indicates that the path is not the same for E. Coli. Despite the fact that glutamic-α-semialdehyde needs only the amination of the terminal aldehyde group to yield ornithine, the data indicate that it is not directly involved in ornithine sythesis by

E. Coli. Acetyl glutamic acid is a more likely intermediate, especially since glutamic-less mutants can utilize this compound showing the ability to split the acetyl group and suggesting the reverse acetylation.

It is, therefore, postulated that

$$\text{L-Glutamic acid} \xrightarrow{\text{(acetylated)}} \text{N-acetylglutamic acid} \xrightarrow{\text{(reduced)}}$$
$$\text{N-acetyle glutamic-}\alpha\text{-semialdehyde} \xrightarrow[\text{(aminated)}]{} \text{acetylornithine}$$
$$\xrightarrow[\text{(deacetylated)}]{} \text{Ornithine}$$

The enzyme required for the deacetylation of acetyl ornithine has been obtained in a cell free preparation and is dependent upon CO^{++} for activation. A second enzyme essential for the amination of acetyle glutamic-α-semialdehyde has also been identified and the reaction shown to be a transamination involving the aldehyde group.

M.-L. J.

BOOK REVIEWS

PORTRAIT OF A HOSPITAL, 1752-1948, TO COMMEMORATE THE BICENTENARY OF THE ROYAL INFIRMARY, MANCHESTER. By William Brockbank. London, William Heinemann Ltd., 1952. 10 + 218 pp., colored frontispiece, 56 illus., and 3 maps. 25*s*.

THE FIRST HUNDRED YEARS OF THE MOUNT SINAI HOSPITAL OF NEW YORK, 1852-1952. By Joseph Hirsh and Beka Doherty. New York, Random House, 1952. xi + 364 pp., 68 illus. $5.00.

The bi-centennial of the great Royal Infirmary at Manchester, and the centennial of the distinguished Mount Sinai Hospital of New York are fittingly and most appropriately commemorated in these books. Both give a remarkably vivid picture of the vicissitudes in the growth and development of a hospital of high standard and achievement, and both are well written, well organized, and attractively illustrated.

Much interest attaches to the Royal Infirmary of Manchester because of its prominence in meeting the extraordinary social crisis involving health affairs in the Manchester area, as a result of the great industrial revolution of the late eighteenth and early nineteenth centuries. The Manchester Infirmary is famed for the extraordinarily high quality of medical service rendered to indigent patients there during the critical turning point in medical development from ancient empirical methods to those dependent on modern scientific fundamentals. From this high standard of medical service came also the important formulation of ethical principles which have been widely influential since, both in the English-speaking Commonwealths and in the United States of America. Highly impressive is the story of extensive destruction suffered by the Royal Infirmary in Manchester during World War II, and the amazing restorations which have taken place more recently.

Doctor William Brockbank is himself carrying forward an honorable tradition in continuing the studies initiated by his distinguished father, Doctor E. M. Brockbank, who has written so well on the early history of the Manchester Infirmary, and also of some of the other hospitals associated with it (*Sketches of the Lives and Work of the Honorary Medical Staff of the Manchester Infirmary,* Manchester University Press; *The Foundation of Provincial Medical Education in England,* Manchester University Press, 1936, and *A Short History of Cheadle Royal,* Manchester, 1934). Highly gratifying to the people of Manchester must be the realization that the Royal Infirmary has been well maintained throughout two centuries by the generosity of generations of their forebears. It is an outstanding example of what medical leadership can be in respect to hospital service on private initiative without governmental support or regulation. Appropriately, the seal of the hospital depicts the story of the Good Samaritan, and to the lesson of this story the people of Manchester have been true.

The scope of the history of the Manchester Royal Infirmary is indicated from the chapter headings of Doctor Brockbank's book—The Lunatic Hospital; Public Baths and Public Health; The Peterloo Calumnies; Extension and the Royal Patronage; The Case of the Absconding Secretary; The Wards are Unhealthy (1836-1851); The Queen's Visit and the Centenary; The Developments and Disappointments; The Great Infirmary Controversy; The Building of the New Hospital; The Two Hospitals at Cheadle;

The Rise of Specialization, and Destruction and Rehabilitation. Particularly impressive are the fine illustrations, the plans, the maps, and the "Rules and Orders of the Public Infirmary at Manchester, 1752" printed as an appendix. There is a good index.

The volume on the Mount Sinai Hospital of New York is equally well organized and illustrated and proceeds in a warm, narrative style to tell the same sort of story, varying only in person and place, regarding the rapid events and excitement of development and service. Organized as "The Jews' Hospital in New York," the institution filled an immediate need in the rough and poverty-stricken metropolis of the new world. Opening on West 28th Street in New York City in a modest four-story building, the Jews' Hospital quickly took its place in caring for the sick of the community and rendered worthy service during the Civil War. Shortly its name was changed to The Mount Sinai Hospital, and it moved to Lexington Avenue and 66th Street. Here it developed one of the best nursing schools in the country at that time. Its staff included some of the outstanding medical leaders in New York, and in spite of expansions and the difficulties of new buildings, it gradually developed the clinics and laboratory services which were to make it outstanding. The staff of Mount Sinai Hospital included such distinguished leaders as Abraham Jacobi, Arpad Gerster, Carl Koller, Bernard Sachs, Henry Koplik, Nathan E. Brill, Emanuel Libman, Leo Bueger, Reuben Ottenberg, Bela Schick, Gregory Shwartzman, and Moses Swick.

In this lively and interesting account of the development of the Mount Sinai Hospital the illustrations are excellent, there is a useful chronology, together with a list of officers and staff, of the Welch and Janeway Lectures, and of endowments and gifts. The volume is well indexed.

For these two volumes commemorating two great hospitals, the respective authors are to be warmly commended. Records such as these provoke astonishment at the long continued devotion of many thousands of persons to the high ideals of medical service. These books give fine examples of what such service can accomplish. Although not as frequently undertaken, the biographies of institutions may be equally as interesting and important as the biographies of single individuals. Institutions, which are made up of changing groups of individuals, may often be successfully molded into agreeable and effective developments by frequent attention to the factors in their growth. Certainly in the case of the Royal Infirmary of Manchester and the Mount Sinai Hospital of New York, those who have been concerned can take pride in the richness of the accomplishment.

CHAUNCEY D. LEAKE

CARBOHYDRATE METABOLISM. A SYMPOSIUM ON THE CLINICAL AND BIOCHEMICAL ASPECTS OF CARBOHYDRATE UTILIZATION IN HEALTH AND DISEASE. Edited by Victor A. Najjar. Baltimore, The Johns Hopkins Press, 1952. 134 pp. $4.00.

A number of experts in various phases of carbohydrate metabolism contributed papers which were presented at a symposium at the Johns Hopkins Hospital, Department of Pediatrics. The subjects covered include biochemical and clinical aspects of glycogen metabolism, the relationship of pituitary hormones to carbohydrate metabolism, and observations on the relationship between electrolytes and glucose utilization both in experimental animals and in man. A very excellent summarizing chapter is in-

cluded in which the editor attempts to synthesize the points of view presented by the essayists. There is a short but adequate bibliography after each essay.

Much of the material that is presented in this book has previously appeared in other publications, but the excellent chapters on glycogen synthesis and molecular configuration by Carl Cori, on the control of liver and muscle phosphorylase by Sutherland, and on glycogen disease by Andersen together make a particularly enlightening unit which constitutes a considerable body of previously unpublished information.

It is, perhaps, not surprising that the essayists have elected to use the same diagrams in the book that they used at the conference, but this sometimes leads to confusion, since it is difficult to estimate the significance of the changes described. This difficulty is actually referred to by Dr. Hastings in his paper when he implies that the results persented were not obtained consistently.

It would have been very interesting if the editor had included the discussion which must have occurred after the presentation of each paper, but the omission of this material can be excused since, according to the preface, the decision to publish these papers was made after the symposium had been concluded.

This collection of essays will be of great use to clinicians interested in the application of fundamental research to the clinical problems of carbohydrate metabolism and should be available in libraries and collections related to this subject.

PHILIP K. BONDY

THE AURICULAR ARRHYTHMIAS. By Myron Prinzmetal, Eliot Corday, Isidor C. Brill, Robert W. Oblath and H. E. Kruger. Springfield, Ill., C. C Thomas, 1952. 387 pp. $16.50.

This volume is a most careful and detailed account of the experimental evidence that the auricular arrhythmias have a common origin in a rapidly discharging focus in the auricle, and that the concept of a "circus movement" as the cause of auricular flutter and fibrillation is invalid.

In the pursuit of their evidence, the authors have amplified and extended the original observations of Sherf on aconite-induced arrhythmias. By the use of special methods, including a new surgical approach to the auricles, direct electrocardiographic exploration of the auricles and the interauricular septum, and especially the technique of clinematography at 1-2 thousand frames per second, the arrhythmias due to aconite, mechanical and electrical stimulation, and of spontaneous nature are analysed much more completely than has heretofore been possible. It is indeed unfortunate that the reproductions of still photographs provide, in the words of the authors, "only a poor conception of the movements of the auricle seen in the (moving) pictures."

The material in the monograph is presented in eighteen chapters and an appendix. Normal auricular activity, premature auricular systoles, paroxysmal auricular tachycardia, auricular flutter and auricular fibrillation are each discussed in relation to their clinical and experimental characteristics, both in man and in the dog. Chapters are included outlining the application of a new method of interpreting electrocardiograms of these arrhythmias, and summarizing current practices in the therapy of these disorders. The

appendix gives the types of equipment and techniques used in the studies upon which the monograph is based. Typography and format are exceptional throughout.

Although there is much in this book which will be of interest to the general reader, there is enough highly technical material to provide the cardiologist, physiologist, and pharmacologist with much new and useful information.

ALLAN V. N. GOODYER

THE URINARY FUNCTION OF THE KIDNEY. By A. V. Wolf. New York, Grune & Stratton, Inc., 1950. xiii + 363 pp. $7.50.

Bold is the renal physiologist who questions today the validity of such concepts as inulin clearance, thresholds, and tubular maxima. Yet Dr. Wolf asserts that the modern theory of renal function, aside from resting on inconclusive fundamental experimental evidence, is misleading as regards the over-all function of the kidney. In place of the usual consideration of the handling of urinary solutes taken independently, that is excretion, the emphasis is here put on the simultaneous disposition of urinary water and solutes for the maintenance of plasma pH, osmotic strength, and volume, i.e., regulation.

The monograph is essentially a presentation of new interpretations, not of new facts. For example, threshold, through a slight modification in definition, takes on a wholly new significance, which renders the concept of maximal tubular reabsorptive capacity meaningless, and removes the dissociation of threshold from the rate of urinary flow. The term isorrhea is introduced to define the state of urinary excretion equal to a rate of loading of salt and water. The upper and lower limits of these rates of loading may be experimentally determined. These are the limits of the regulatory function of the kidney and are different from the simple ability to concentrate or dilute.

There is commendably accurate definition of such nebulous words as diuresis and dehydration and there is strict adherence to these definitions throughout the text. The exceptionally lucid chapter on the dehydrations owes its excellence especially to the careful use of terms.

The distinctions between the points of view presented here and those of Homer Smith are not always clear-cut. That the performance of the kidney in the last analysis must be considered as a function of *all* the attributes of the presented plasma, of course, cannot be disputed. This monograph seems in many instances to go no further than putting old problems in new terms. In this much at least, the unorthodox approach is refreshing and stimulating.

W. P. McN.

MICROSCOPIC HISTOCHEMISTRY PRINCIPLES AND PRACTICE. By George Gomori. Chicago, University of Chicago Press, 1952. 273 pp. $6.00.

Gomori's "Microscopic Histochemistry" is the first extensive critique in English devoted exclusively to histochemical methods. Its scope includes methods for localization of inorganic substances, metals and non-metals, organic substances including saccharides, lipids, proteins and their derivatives, and finally oxidative and hydrolytic enzymes. The chapters are well documented and there is also a section of many additional references, classified according to subject, which should be very useful. The methods are

presented in sufficient detail to obviate in most instances reference to the original article, if indeed the particular procedure did not originate with, or was not modified by, Gomori himself. There are forty references to his work and most of these describe techniques he has introduced or studied.

The author is known for his critical faculty. This is given full expression in the present work. Those who are new in the field will find this valuable, although its effectiveness is sometimes lost, especially when a particular method, as far as can be ascertained, appears to be criticized because of assumption rather than through actual experience. The section on the detection of inorganic iron is essentially the same as that presented in his excellent article of 1936 (Am. J. Path., *12,* 655) and certain of the assertions might be somewhat revised in view of the findings of others since that time.

The book is a valuable one and should form part of the reference library of a histochemical laboratory.

HENRY BUNTING

HUMAN EMBRYOLOGY. By William J. Hamilton, James D. Boyd and Harland W. Mossman. Baltimore, The Williams and Wilkins Co., 1952. viii + 432 pp., 433 figs., 5 tables. $9.00.

This widely used, valuable textbook on "Human Embryology" has now appeared in an extensively revised second edition much of which has been completely recast and rewritten. Some of the original figures in the first edition have been redrawn or replaced and 69 new ones have been added, making a total of 433 excellent illustrations. Many of them are in color. In fact the high quality and selection of the beautiful illustrations which accompany the clearly written text, all on a superior type of paper, make this the best textbook on human development yet prepared in the English language for medical students. Both the authors and the printers are to be congratulated for producing this fine publication.

There are sixteen chapters, each with a brief introductory statement to orient the reader. To aid him when referring to special sections the headings of the subdivisions are set in heavy type and there is a complete index of twenty-one pages at the end of the book.

Following the introductory concepts of embryology there is an excellent account of the formation and structure of the germ cells and the cyclic changes in the genital tract related to hormonal influences. The descriptions of fertilization and early formation of the mammalian egg and embryo include the newly discovered two-cell human egg and the latest of human material now available. Correlated with this material there is also a valuable table appended at the end of the book listing the important early embryos under twenty-one days of age, what their ages and dimensions are, and references to the publications describing them, giving authors and dates.

Throughout the book emphasis is placed upon the three-dimensional picture with the use of a rich collection of beautiful half-tones chosen at critical levels. Many chapters are devoted to the development of the various organ systems, covering both normal structures and the common anomalies found in man. A brief discussion is also given of the experimental and other factors controlling and modifying normal and abnormal development.

There is a final valuable chapter on comparative vertebrate development with many colored plates showing the chief similarities and differences in the early development of various classes of vertebrates. The formation of the foetal membranes in the higher groups of vertebrates is briefly com-

pared, and the successive stages of implantation and placental formation in various mammals are compared with those in man. Although the authors have quite successfully attempted to keep the book as short as possible, they have succeeded in putting much of great value in it with the result that it can be highly recommended. No biological library is complete without a copy of this excellent account of human development.

L. S. STONE

CYBERNETICS. Transactions of the Seventh Conference, March 23-24, 1950. Heinz von Foerster, Ed. New York, Josiah Macy, Jr., Foundation, 1951. 251 pp. $3.50.

The Macy conference on cybernetics brings together participants from unusually diverse fields. Mathematics, physics, physiology, neurology, psychology, psychiatry, zoology, and anthropology are all represented in the twenty-four members and guests. The text is a transcript of the papers presented plus the discussions; in fact, the latter occupy most of the book. Following an initial paper on digital notions in the central nervous system, the remainder of the presentations are devoted to language—its intelligibility, meanings, and uses.

As might be expected, the result is spotty. The cross-fertilization at times gives brilliant results. At other times it is clearly evident that the participants do not understand one another, and there is little hope for the reader. The problem of communication among members of different disciplines is nowhere more evident.

The title should not be forbidding to one with little mathematical preparation, as virtually no mathematical formalism is included. The style is discursive and digressive, and indeed one wonders in parts where cybernetics fits in, at least in the sense of Wiener. But this does not detract from the fascinating quality of several of the papers, especially those by J. C. R. Lickider and Margaret Mead.

W. P. McN.

PROBLEMS OF CONSCIOUSNESS. Transactions of the First Conference, March 20-21, 1950. Harold A. Abramson, Ed. New York, Josiah Macy, Jr., Foundation, 1951. 200 pp. $3.00.

This volume records an interesting addition to the Josiah Macy, Jr., Conferences on the Science of Man. Five members of the conference presented papers for discussion by the group of twenty-one participants from various fields of biology and medicine.

Nathaniel Kleitman's report on "The Sleep-Wakefulness Cycle" emphasizes the variations in consciousness from deep, dreamless sleep to the highest level of alertness, and the effect of sleep pattern upon cyclic changes in the individual. Discussion is provoked concerning the relative effects of afferent stimulation and a reticular formation activating system in the maintenance of wakefulness.

Gregory Zilboorg presents a straightforward account of "Psychoanalytic Concepts of Sleep and Dream," or at least of dream, for he states that Freud "did not work out any question of sleep." The cathectic theory of consciousness and the effect of socialization are considered in the discussion period.

Henry K. Beecher ("Perception of Pain and Some Factors That Modify It," reports experiments on the efficacy of morphine, pentobarbital, and saline in alleviating post-operative pain. His subjects' reports suggest a useful differentiation between simple "pain" and "suffering." There is discussion of the effects of lobotomy on pain, and the pain perception of psychotics.

Margaret Brenman in "The Phenomena of Hypnosis" suggests six psychological dimensions which distinguish hypnosis from the waking state and offers an explanation for the spontaneous shifts in depth of hypnosis that are often observed. Her presentation occasions comparison of the hypnotized individual with the patient with frontal lobe dysfunction.

"Consciousness and the Chemistry of Time" are considered by Hudson Hoagland. The Arrhenius equation relating chemical reaction-time to temperature is shown to be applicable to the changes in time perception and rate of tapping in people with artificial or pathological fevers. This suggested biochemical basis for the sense of time permits new speculations concerning the effects of age, drugs, and other factors upon time perception.

It is not in the reviewer's province to estimate how useful the conference may have been to its members; some of them seemed to have learned quite a bit. To the reader, however, there is essentially nothing in the presentations that cannot be found in the literature, and no astounding insight is offered in the discussion. The fascination inherent in any verbatim account of conversation is here enhanced by the alternative, and sometimes simultaneous, use of physiological and psychological levels of discourse; it may be that this book will be more useful to the student of communication than to the student of consciousness.

W. A. WILSON

FRONTIERS IN MEDICINE: THE MARCH OF MEDICINE, 1950. New York Academy of Medicine. New York, Columbia University Press, 1951. x + 150 pp. $2.50.

This collection of a series of lectures designed primarily for the layman will appeal to the members of the various medical and biological disciplines as well.

There are six papers contained herein—"Frontiers in Psychiatry" by Franz Alexander; "Methuselah: Myth or Promise?" by David Seegal; "Frontiers in Genetics" by Laurence H. Snyder; "Machines That Work Like Men" by John H. Gibbon, Jr.; "Biological Aspects of Antibiotics" by Selman A. Waksman; and "Concepts and Methods of Medical Research" by Thomas M. Rivers.

Dr. Seegal presents a review of the advances in medicine which have been effective in extending the life expectancy of man and considers the possibilities of future advances in medicine which will permit us to extend our life spans even further. Dr. Gibbons' paper is a description of the heart-lung machine which he and his department are developing at Jefferson Medical College. These two papers are aimed directly at the interested layman and are to be so recommended.

Dr. Waksman presents the historical data, discusses the nature and formation of antibiotics, their mode of action, their place in the ecology of micro-organisms and also presents a list of ten criteria which a new antibiotic should meet before it should be considered as a new chemotherapeutic agent. Dr. Rivers concerns himself with the various methods of medical

research and evaluates the advantages and disadvantages of co-operatively organized, institutional research programs as opposed to individual research programs.

"Frontiers in Psychiatry" is concerned with the place of psychiatry in education and sociology, as well as international relations. This superior presentation demonstrates the present-day limitations of psychiatry but also points out how psychiatry, properly utilized, may, in the future, help us to plan our political organizations and the educational programs of our children to facilitate the achievement of our socio-political aspirations. Dr. Alexander also discusses some of the fallacies in the Marxian concepts of man's psychology.

"Frontiers in Genetics" by Dr. Laurence Snyder is one of the finest papers of its type that this reviewer has read. Dr. Snyder presents a thorough, competent, and broad review of the relationship of genetics to the other sciences. He propounds an impartial, honest, and intelligent view of the present Michurin vs. Mendelian genetics controversy. Superlatives should be used sparingly, but certainly Dr. Snyder has approached the ideal in this paper. It is recommended for the college biology student, the interested layman, medical students and practitioners, and the graduate student of biology.

There is a foreword by Dr. H. B. Keyes and an introduction by Dr. Iago Galdston. The book is adequately indexed and there are bibliographies at the end of each paper. This little volume is unquestionably worth the nominal cost of $2.50.

J. M. Q.

CARBOHYDRATE METABOLISM. CORRELATION OF PHYSIOLOGICAL, BIOCHEMICAL AND CLINICAL ASPECTS. By Samuel Soskin and Rachmiel Levine. Chicago, University of Chicago Press, 1952. 2d ed. 346 pp. $8.50.

The authors have revised and expanded the first edition of this work, published in 1946, so that a good deal of the recently published research in carbohydrate metabolism and allied problems is included. Since they have elected to do this without altering the general approach of the original edition, the table of contents, the figures, and the bibliographies are virtually unchanged. New material has been added, where necessary, usually by appending an extra sentence or paragraph to the previous discussion; but, in many instances, whole paragraphs have been completely rewritten. For the most part, this type of editing has been successful, but in some cases the new material has been too obviously tacked on to the old so that the text does not always flow smoothly. In one or two instances, actual contradictions occur in the text because only one of two statements has been modernized. For example, on page 122, the authors alter the statement in the first edition that acetone is a spontaneous breakdown product of acetoacetate by pointing out that acetone may also be derived directly from valine by a "physiological" process. One paragraph later, however, they fail to correct the statement that "Acetone . . . is merely a spontaneous decomposition product which indicates that an equivalent amount of one of the other ketone bodies was formerly present."

In spite of these minor criticisms, the book is a lucid and emphatic statement of the authors' viewpoint regarding carbohydrate metabolism. The crucial points of the argument revolve about the rôle of the liver and the regulation of the rate of entry of glucose into the intracellular space. In discussing these matters, the authors bring very strong and constructive

criticism to bear upon certain outmoded and diffuse concepts such as the respiratory quotient as an index of the composition of the metabolic mixture, and the use of the D/N ratio as a criterion for complete diabetes. These techniques had been used in the past to prove that carbohydrate utilization is reduced in diabetes. Since the authors feel that the R.Q. and D/N ratio are not helpful in determining the rate of utilization of carbohydrate, they are free to expand upon the possibility that the regulation of carbohydrate metabolism is largely a result of alterations in the rate of production of glucose by the liver. This occurs, presumably, chiefly by changes in the rate of conversion of fat to carbohydrate.

The conclusion the authors draw from this hypothesis is that peripheral glucose utilization is not greatly altered under most circumstances where alterations of carbohydrate metabolism occur. According to this theory, in diabetes mellitus or after the administration of insulin, the alterations of blood glucose and the changes of fat and protein metabolism are entirely a result of changes in the rate of production of glucose by the liver. Even the hormones of the adrenal cortex are considered as having their action chiefly by regulating liver function.

This line of reasoning forces the authors to accept certain logical inconsistencies. The idea that diabetes mellitus is a result *only* of overproduction of carbohydrate from fat seems to do violence to certain recent isotope studies which show that utilization of carbohydrate is also impaired. For example, the authors quote Feller, Chaikoff, Strisower, and Searle (J. Biol. Chem., 1951, *188,* 865) as proving that the rate of carbohydrate "disappearance" or "utilization" is the same in pancreatectomized as in normal dogs, although the conversion of CO_2 is reduced in diabetes. In rats, Feller, Strisower, and Chaikoff (J. Biol. Chem., 1950, *187,* 571) are quoted as showing that both utilization of glucose and its conversion to CO_2 are undiminished in diabetes. In both experiments, however, the blood glucose concentration was greatly elevated in the diabetic animals as compared with the normal ones. According to the authors (pp. 163-165) this should produce an *increase* in carbohydrate utilization. The fact that diabetic animals with increased blood glucose concentrations have depressed or normal glucose utilization indicates that, considering the blood glucose concentration, there *is,* indeed, depression of carbohydrate utilization. This type of evidence is particularly important in evaluating the authors' viewpoint because it is completely independent of the R.Q. and D/N ratios.

The chief criticism of the book is not that it presents unreasonable ideas, or that the conclusions reached by the authors are necessarily incorrect; but rather, that the theories and hypotheses *are,* at present, still unproven. The uninformed reader might fail to appreciate this fact, under the impact of the forceful presentation, and of the authors' ideas and their strong rejection of alternative interpretations. Since the book is clearly intended to serve as an introduction to carbohydrate metabolism, it will appeal chiefly to readers who are not entirely familiar with the field. It is this group who are most likely to be misled by the authors' enthusiasm. In this reviewer's opinion, therefore, the book can be recommended only as an excellent presentation of a single point of view regarding carbohydrate metabolism. It would be misleading to consider the interpretations presented in this monograph as the only possible ones. But with these reservations, the book can be recommended to readers who want a consideration of the problem which is refreshingly different from the traditional one.

PHILIP K. BONDY

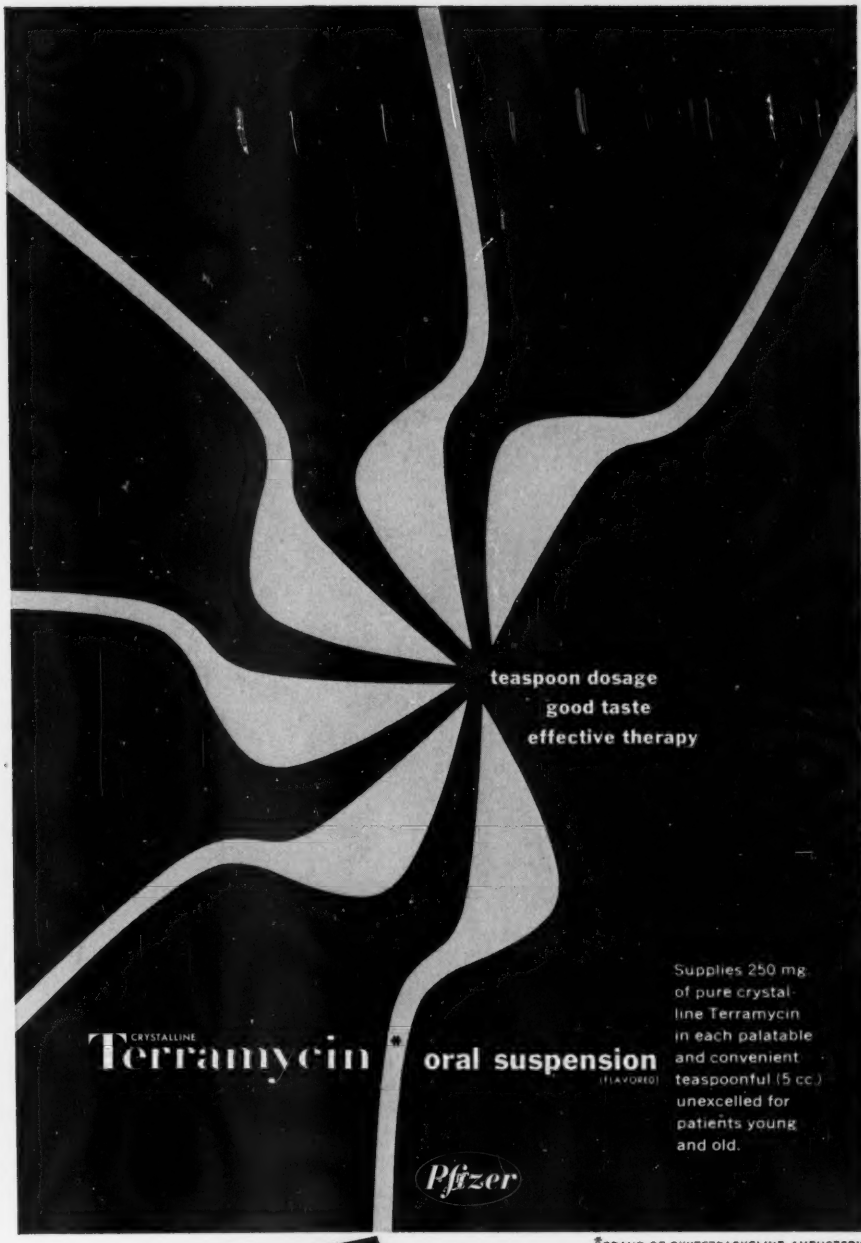
PROBLEMS OF LIFE, AN EVALUATION OF MODERN BIOLOGICAL THOUGHT. By Ludwig von Bertalanffy. New York, John Wiley & Sons, 1952. 216 pp. $4.00.

This book is a translation of *Das biologische Weltbild,* vol. I, which was published in Berne (Switzerland) in 1949. L. von Bertalanffy is well known to biologists as one of the leaders in the attempt to develop biology as an autonomous science with its own laws. This attempt gives to the organic sciences perspectives of thought which seem often to have been lost by the one-sided influence of the inorganic sciences which developed earlier. The necessity for a reorientation was clearly stated and analysed in the theory of stratification of reality in the ontology of Nicolai Hartmann.

The approach chosen by the author and developed and elaborated in broad outlines is the so-called organismic concept. This is contrasted with vitalism and mechanism in an attempt to solve this age-old dispute. The organism is here conceived as a system which is a complex of elements in mutual interaction, this system having a dynamic hierarchial order and an autonomy of its own. The book is fundamentally an elaboration of this conception of the organism. This theory of dynamic structure of living organisms and the interpretations and schemes derived from it are of extreme importance for every branch of biology. It permits a description of biological organization in scientific terms, i.e., laws governing organization within living matter. The author describes biological phenomena at all levels of organization (physico-chemical, cellular, organismic, and communal) strictly within the framework provided by this concept. The examples chosen are taken principally from zoology.

The book is written in sufficiently broad terms to be easily comprehensible. The reader who desires a more specific and exact language should consult the author's *Theoretische Biologie,* volumes I and II. The present book is an important one and one hopes it will find the place it deserves in the development of biology. The author shows clearly that biology is not an accumulation of facts but rather a constant re-interpretation of the problems of organic systems.

KRAFT VON MALTZAHN



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